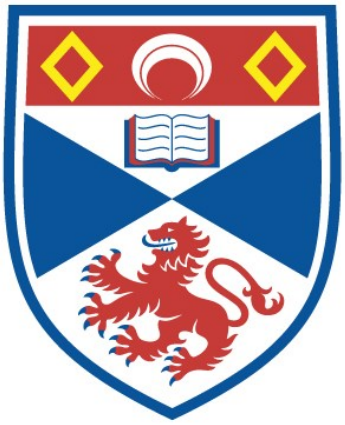
CHANGES INDUCED IN MITOTIC INDICES OF ROOTS OF VICIA FABA L. BY COLCHICINE AND IAA

Ronald Dorward MacLeod

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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Changes induced in mitotic indices of roots of *Vicia faba* L. by colchicine and IAA.

by

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A thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy.

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Declaration.

I hereby declare that the following Thesis is based on the record of work done by me, that the Thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The research was carried out in the Department of Botany at St. Salvator's College of the University of St. Andrews under the direction of Professor J.A. MacDonald and Dr. D. Davidson, and, at the Botanisches Institut of the University of Tübingen, in the laboratory of Herr Professor Dr. E. Bünning.

Certificate.

I certify that Ronald Dorward MacLeod has spent nine terms of research work under the direction of Dr. D. Davidson, Herr Professor Dr. E. Bünning and myself and that he has fulfilled the conditions of Ordinance No. 16 (St. Andrews), and that he is qualified to submit the accompanying Thesis in application for the degree of Doctor of Philosophy.

Acknowledgements.

I wish to record my gratitude to Professor J.A. MacDonald and Dr. D. Davidson of the Department of Botany, St. Salvator's College, for supervising the work presented in this Thesis, and for the deep interest they have shown throughout the investigations.

I am also indebted to Herr Professor Dr. E. Bünning of the Botanisches Institut at Tübingen for offering me the hospitality of his laboratory. I should also like to thank the Science Research Council for a grant without which these investigations could not have been undertaken.

Career

I graduated from the University of St. Andrews, in June 1963, with first class honours in Botany.

In October 1963, I was admitted as a Research student in the University of St. Andrews under Ordinances 16 and 61 and studied there until December 1964. In January 1965 I studied at the Botanisches Institut in Tübingen under the supervision of Herr Professor Dr. E. Bünning. I returned to St. Andrews in July 1965.

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INTRODUCTION

Eclectic

Introduction

The root apex consists of a subterminal meristem and the root cap. The meristematic cells surround a cup-shaped region of cells which rarely synthesise DNA (deoxyribonucleic acid) and seldom divide. They constitute the quiescent centre (Clowes, 1959). The surrounding meristematic cells, however, actively undergo DNA synthesis and division.

IAA (B-Indole-3-acetic acid) is present in many plants (Leopold, 1955; Audus, 1959), including Vicia faba (Bennet-Clark and Kefford, 1953), and has been implicated in mitotic cycles (see Discussion). Pilet (1951) found that the greatest concentration of IAA in roots of Lens culinaris is in the meristematic zone of the root apex. The concentration of IAA decreases with increase in distance from the apex.

When plants are irradiated, there is a decrease in the endogenous auxin level, as IAA biosynthesis is very sensitive to X-rays, though IAA itself is relatively insensitive (Gordon, 1956). Gray and Scholes (1951) exposed roots of Vicia faba to 140 r of ionising radiation. When the apical meristem of the primary root was irradiated growth was inhibited. Thousands of roentgen are needed to affect root elongation if the meristem is protected (Gray and Boag, unpublished, quoted by Howard and Pelc, 1953).

IAA, depending upon the concentration used, can stimulate or inhibit root growth by elongation (Bonner and Koepfli, 1939; Lundegårdh, 1942; Burström, 1942; Street et al, 1954). It has been suggested therefore, that IAA is synthesised in the root apex (Davidson, 1960; Torrey, 1963). It is probable that some of the IAA synthesised in the root apex moves basally in the root and is involved in root elongation. X-irradiation thus appears to affect root elongation by blocking the synthesis of IAA in the root apex.

Concentrations of IAA, which are inhibitory to root elongation, may induce the formation of sub-apical root tumours (Levan, 1939). Such tumours develop as the result of a changed polarity of cell expansion in the zone of root elongation (Hawkes, 1942). Swellings are also formed in intact plants exposed to ethylene (Michener, 1938). It appears that ethylene increases the sensitivity of the tissues to IAA. Thus there is evidence that tumour formation is affected by changes in the amounts of, or in the sensitivity of tissues to, IAA.

Roots treated with the alkaloid colchicine cease elongating and sub-apical swellings, the so-called c-tumours, form (Levan, 1938). These swellings form in the zone of root elongation, when

the cortical cells, which normally elongate parallel to the longitudinal axis of the root, expand isodiametrically (Hawkes, 1942). Thus colchicine affects the root in such a way that a change in the polarity of cell expansion occurs. This change in polarity is not visible immediately but occurs one to two days after the end of treatment. It has already been shown that IAA is implicated in the polarity of cell expansion and it therefore seemed possible that the changes in the pattern of growth of roots treated with colchicine were due to a change in the level of auxins in the roots. Roots treated with 10^{-8} M or stronger solutions of IAA show inhibition of growth (Bonner and Koepfli, 1939; Duhamet, 1945). In roots previously treated with colchicine, however, we have shown that 10^{-6} M IAA stimulates growth (Davidson, MacLeod and Taylor, 1965). This effect supports the suggestion that roots treated with colchicine undergo a change in the level of IAA. Such a change could account for various abnormalities seen after colchicine treatment, e.g. reduction of root growth (Levan, 1938; Hawkes, 1942; Davidson, 1961, 1965) or aberrant patterns of xylem formation (Davidson, 1963). The changes in xylem pattern in colchicine treated roots appear to follow changes in IAA levels in view of the report that xylem formation is controlled by IAA (Torrey, 1957).

IAA can stimulate non-dividing cells to enter mitotis, for example, pericycle cells (Goldacre, 1959) and mature cells of roots (Levan, 1939; D'Amato, 1945), and it is necessary for mitosis and DNA synthesis in tissue explants (Patau, Das and Skoog, 1957). In fully formed meristems, however, IAA appears to inhibit mitosis, even in concentrations as low as 3 ppm (Stern, 1958).

In plant tumours there is also evidence that meristematic cells are the site of IAA synthesis and that, like root meristematic cells, they are sensitive to radiation. Link and Eggers (1941) found that there was more auxin in tomato crown-gall tumour tissues than in the corresponding normal stem tissues. The tumour cells themselves are the primary source of IAA (DeRopp, 1947). The growth of such tumours is slowed down by irradiation and this inhibition is reversible by the addition of IAA (Klein and Vogel, 1956). Because tumour growth is entirely due to the duplication of tumour cells, this suggests that IAA is probably necessary for tumour cell division.

The nuclei of normal lateral bud promeristems of Tradescantia, which are inhibited by the main bud, have only the 2c amount of DNA (Naylor, 1958). Two to three days after removal of the terminal bud, however, they had the 4c amount of DNA,

and four to five days after decapitation the cells of the lateral bud promeristems underwent cell division; they eventually developed into lateral buds. If NAA (naphthaleneacetic acid), a similar compound to IAA, was put on to the decapitated stem immediately the terminal bud was removed, there was no change in the DNA content of bud promeristem nuclei, and no cell division or development occurred. This is clear evidence that events in interphase nuclei can be controlled by auxins. Naylor's results show that the dormant condition of the promeristem nuclei was controlled by a supply of auxin from outside the cells. These results, from tumours and decapitated shoots, suggest that auxin is involved in the control of mitotic cycles. It is apparently necessary for cell replication though in high concentrations it exerts an inhibitory effect.

Irradiation of Vicia faba roots has been shown to result in a fall in the MI (mitotic index) and in the rate of root growth (Gray and Scholes, 1951; Clowes, 1959). Though most of the cells of the apical meristem stop dividing, some cells remain capable of active division and, a new meristem is formed in the quiescent centre (Clowes, 1959). Division of the quiescent centre cells following irradiation is seen at the time when root growth has stopped (Davidson, 1960). These results from

irradiated roots pose a problem that is central to our understanding of the organisation of root apical meristems; why do some cells in the root apex begin active division only after a treatment that appears to inhibit division of other cells?

Colchicine upsets normal mitosis in root apices by preventing the anaphase separation of the chromatids (Levan, 1938). This appears to be a consequence of the dissolution of the spindle apparatus (Inoué, 1952). Following anaphase inhibition there is an increase in the MI due to the accumulation of cells in metaphase. These cells arrested in metaphase undergo an aberrant form of mitosis, known as c-mitosis (Levan, 1938), form restitution nuclei and produce polyploid cells.

Roots of Vicia faba have been treated with colchicine and their growth and meristem constitution followed for several days (Levan, 1938; Davidson, 1961). The meristems, which form after treatment, contain both diploid and polyploid cells. Diploid cells are found in division three days after colchicine treatment, mainly, as Levan (1938) pointed out, at the apex. The quiescent centre cells would be expected to remain unaffected by colchicine since they are not in division during treatment. On the basis of results from irradiated roots (Clowes, 1959) and results from roots growing several days after colchicine treatment (Davidson,

1961), it seems probable that following a treatment with colchicine, cells of the quiescent centre synthesise DNA, come into division and contribute to the formation of a new meristem. The results from colchicine treated roots parallel those found after irradiation: root growth is inhibited, the MI of the general meristem falls and division is seen in diploid cells at the apex of the root. Since these effects can be associated with a change in auxin level when roots were irradiated it seemed possible that the effects induced by colchicine were due at least in part to an alteration in auxin synthesis. The implications of this suggestion have been tested by treating roots with colchicine and IAA, either together or in succession.

The experiments to be reported here were undertaken to determine whether:

1. The effect of colchicine on spindle disruption is reversible by IAA.
2. IAA affects the delay at metaphase induced by colchicine, i.e. does it effect intermitotic time?
3. Colchicine affects the MI apart from the increase due to metaphase delay, i.e. does it effect intermitotic time?
4. The change in polarity demonstrated by isodiametric expansion of cortical cells in colchicine treated roots is also reflected in a change in the orientation of the spindle axis of the dividing cells.

5. Treatment with colchicine affects the location of the first formed xylem elements and lateral root primordia.

SECTION ONE

THE EFFECTS OF COLCHICINE AND IAA ON MITOSIS

Introduction

The evidence already given is indicative of a great similarity in the effects of colchicine and IAA on several aspects of root growth. However, though cells actually undergoing mitosis are known to respond to colchicine, no evidence has been obtained that IAA affects the course of mitosis. Thus, though the immediate response of cells to IAA and colchicine differs, there is evidence that they respond in a similar way one to two days after treatment, i.e. both compounds induce a change in the polarity of cell elongation. This evidence together with the reports that disruption of the meristem leads to a fall in auxin synthesis led us to consider whether any of the delayed effects of colchicine, i.e. those found many hours after treatment, were due, at least in part, to a change in auxin balance in the roots.

It was decided to determine whether spindle disruption induced by colchicine was reversed by IAA. Roots were treated with mixtures of colchicine and IAA. There was no evidence that IAA prevented the colchicine induced disruption of the spindle. The experiments on the effects of mixtures of IAA and colchicine included treatments with HCl in order to determine

whether any possible effect of IAA could be due to pH change. Roots were treated with mixtures of IAA and colchicine and also colchicine and HCl. The results indicate that there is no immediate interaction between IAA and colchicine, or that the effect of colchicine seen after a three hour treatment is modified by a change in pH.

In addition to examining roots immediately after the end of a three hour treatment they were also examined after a recovery period of twenty-four hours. This period of recovery was chosen for two reasons: first, because this is the time, after a three hour treatment, when cells begin to show a change in polarity; and secondly, it is the average duration of a mitotic cycle. At the end of this recovery period it was clear that IAA could modify the response of the roots to colchicine. Two changes were detected: one was a change in MI and the other was a change in the frequency of tetraploid cells seen in division.

1. Colchicine and pH

Materials and Methods

Beans (Vicia faba) were soaked in distilled water for 24 hours. The testas were removed and the beans planted in moist sand. When the radicles were about 6 cm. long the beans were washed free of sand and grown in water containing complete Hoagland's nutrient solution. The beans were illuminated continuously. The culture solution was aerated and changed every 36 hours. All treatments were carried out at 20° C.

Roots were treated for three hours with:

(i) 0.025% colchicine;
(ii) 6.26×10^{-4} M IAA;
(iii) 10^{-4} M HCl;
(iv) a solution containing 0.021% colchicine and 6×10^{-3} M HCl;
(v) a solution containing 0.021% colchicine and 1.04×10^{-4} M IAA; or,
(vi) left to grow on in the culture solution.

Lateral roots were fixed at the end of the three hour treatment in 1/3 (v/v) acetic acid - absolute ethyl alcohol with one drop of formalin. They were hydrolysed in N HCl at 60° C for eight minutes, stained in Feulgen and the apical meristems prepared as permanent squash preparations (Darlington and La Cour, 1960). Ten slides were made for each treatment. From each slide 600 cells were scored, at random, giving a total of

6,000 cells scored for each treatment. pH readings were also taken for all the treatment solutions used.

Results

Samples of 6,000 cells were scored for each treatment and the number of cells in division determined. From this data, the percentage frequency of cells in the various phases of mitosis was found (Table 1). No significant differences were found, in the percentage frequency of cells in prophase and metaphase, between treated roots or roots grown in the culture medium. Changes in the numbers of cells in prophase and metaphase following the different treatments are not pronounced. There is a slight increase in the number of cells in metaphase in the roots treated with colchicine or with the mixture containing colchicine and IAA, though in the latter case the increase was less marked. This increase in the number of metaphases is a result of colchicine blocking the anaphase separation of the chromatids, leading to metaphase delay.

No true anaphase figures were seen in any of the roots treated with either colchicine, or with the mixtures containing colchicine and either IAA or HCl. Abnormal anaphases and telophases with bridges were observed in the cells of the roots treated with colchicine, though they were less frequent than in controls

(Table 1). This indicates that, within a three hour treatment, neither IAA nor HCl has counteracted the effect of colchicine, in preventing the anaphase separation of the chromatids. Thus, from the frequency of mitotic stages, there appears to be no immediate interaction between colchicine and either IAA or HCl. The effect of colchicine on the spindle is not simply due to a change in pH, since the pH of a mixture containing colchicine and either IAA or HCl is lower than that of the colchicine solution (Table 1).

IAA itself does not suppress anaphase at the concentrations used. However, the chromosomes were seen to be 'sticky' and numerous anaphase bridges were evident. HCl has no apparent effect on the anaphase separation of the chromosomes.

Two conclusions can be drawn from this experiment: first, the effect of colchicine in preventing spindle formation is not a pH effect, and secondly, IAA does not appear to be a factor involved in spindle formation.

2. Colchicine and IAA; Effects on MI

Materials and Methods

Beans were grown as before. Roots were treated with either 0.025% colchicine and, or, solutions of IAA, varying in

concentration from 0.329 to 6.26 x 10^{-4} M; other beans were grown in the culture medium. Lateral roots were fixed either at the end of the three hour treatment, or after a recovery period of 24 hours.

Ten permanent squash preparations were made for each treatment. From each slide 600 cells were scored at random, i.e. 6,000 cells were scored for each treatment.

Results

1. Treatment for three hours

A. IAA

It can be seen (Table 2; Figure 1) that the MI was not altered significantly by any of the treatments with IAA. The values for MI after treatment with IAA ranged from 3.3 to 5.3 compared with the control value of 4.6. There was also no detectable effect on the relative frequencies of cells in the various stages of mitosis (Table 3). It appears that with the concentrations used here, a three hour treatment with IAA had no effects on either the entry of cells into mitosis or on the rate at which cells pass through the various stages of mitosis.

Roots from only one treatment, 2.083 x 10^{-4} M IAA, showed a visible response to IAA. Their chromosomes were highly contracted and were between 1/8th and 1/16th of the length of normal

chromosomes. This effect of IAA on spiralization was not found, however, in other treatments.

B. Colchicine

A three hour treatment with 0.025% colchicine results in an increase in the MI over the control value (Table 2; Figure 1). The difference between the MI of the control roots and those treated with colchicine was not significantly different (Table 4). Thus a three hour treatment with 0.025% colchicine has increased the MI, but the increase is not significant.

Within the period of treatment, however, colchicine has produced the expected changes in the cells: they are arrested at metaphase, leading to the disappearance of anaphase and telophase stages, and producing an increase in the number of metaphases (Table 2; Figure 2). This build up of metaphases reflects the temporary delay, induced at this stage, by colchicine. The increase in the number of metaphases in roots treated with colchicine as compared with control roots is highly significant ($p = < 0.001$). Thus, though the MI is not changed significantly, the frequency of cells in metaphase is changed significantly.

C. Colchicine and IAA

It can be seen (Table 2) that none of the changes induced by colchicine are modified by IAA within a three hour period of

treatment. Even in the presence of IAA, colchicine continues to induce an increase in MI, to suppress anaphase and telophase stages and to increase the number of metaphases.

Comparisons were made of the difference in MI between roots treated with colchicine or the solution containing colchicine and IAA, and also between the controls, grown in the culture medium, or in the solution containing colchicine and IAA. The difference was not statistically significant in either case (Table 4). Therefore, within a three hour period of treatment, IAA has not modified any effect of colchicine on the MI of the roots.

However, it is evident (Figure 1) that the MI of the control roots and of the roots treated only with IAA differ from the MI of roots treated with colchicine or the mixtures containing both colchicine and IAA, since the mitotic indices of the former are always lower than those of the latter. No anaphase figures are present in the roots treated with the mixtures containing colchicine and IAA. It appears that the effect of colchicine on the mitotic spindle is not reversible by IAA and, therefore, that its effect is not due to an inhibition of some process normally dependent upon IAA.

2. Treatment for three hours and 24 hours recovery.

A. IAA.

Within a three hour period IAA, as we have seen, has no significant effect on the MI of the treated roots. However, 24 hours later marked effects can be detected. MI has been plotted against concentration of IAA (Table 5; Figure 3). Only the lowest concentrations of IAA used have no effect on MI; those of 3.13×10^{-4} M to 6.26×10^{-4} M IAA reduce the MI. In three groups of roots the MI was less than 1, compared with a control value of 6.95 (Table 5). One of the low values of MI, in roots treated with 4.2×10^{-4} M IAA, was compared with the control and the difference between their mitotic indices was highly significant ($p = 0.001$; Table 4). Thus, though there is no detectable effect on MI immediately following a three hour treatment with IAA, there is an effect eventually : 3.13×10^{-4} M -- 6.26×10^{-4} M IAA lead to inhibition of mitosis (Table 5; Figure 3).

With the exception of the roots treated with a 6.26×10^{-4} M solution of IAA, IAA does not affect the relative frequencies of cells in the various mitotic stages (Table 6). This suggests that IAA does not interfere with the rate at which cells move through the various stages of mitosis. However, since the higher concentrations of IAA reduce the MI (Table 5), IAA must slow down

some process or processes during interphase. We see, therefore, that although IAA has no effect on MI after a three hour treatment period, there is an effect after a further 24 hour period of recovery. It appears that IAA has no effect on mitosis, but does have an effect in interphase.

B. Colchicine.

The effect of colchicine, 24 hours after treatment ended, has been to raise the MI to 17.2% (Table 5). The difference between this value and that of the controls (6.95%) is highly significant ($p = 0.001$; Table 4). For several reasons it can be said that this increase in MI is not due solely to a delay in metaphase: first, some of the chromosomes held in metaphase begin to revert to the interphase condition three hours after treatment with colchicine was begun, secondly, tetraploid cells, which are the product of restitution, are seen in division 24 hours after treatment, and thirdly, the increase is not due to a wave of synchronised tetraploid cells entering mitotis together (Table 7). Thus, the increase in MI appears to be a genuine stimulation of cells during the period of interphase that follows the colchicine treatment.

Colchicine appears to have had no significant effect on the number of cells in prophase. However, there has been a great

increase in the numbers of cells in metaphase (Table 5; Figure 4), and in the percentage of metaphases (Table 6). Even after 24 hours recovery colchicine treatment has led to the virtual disappearance of cells in anaphase and telophase.

The difference in the number of cells in metaphase, 24 hours after treatment, between the roots treated with colchicine and the controls was tested statistically. The difference was found to be highly significant ($p = < 0.001$). Therefore, the effect of colchicine in blocking the anaphase separation of the chromatids and holding cells at metaphase persists even 24 hours after treatment ended.

The prophase : metaphase ratio in normal roots is about 3.5:1. After colchicine treatment this ratio changes to about 0.3:1 (Table 5). This difference in prophase : metaphase ratio was tested statistically, and was found to be highly significant ($p = 0.00006$; Table 8).

C. Colchicine and IAA.

The effect of IAA in reducing the MI (Table 5; Figure 3) was repeated in roots that had been treated with a mixture of colchicine and IAA and the effect was found to increase with increasing concentration. At the highest concentrations of IAA ($5.2 - 6.26 \times 10^{-4}$ M IAA) the MI was 1 or less. Colchicine,

therefore, does not suppress the inhibitory effect of high concentrations of IAA on the mitotic cycle and mitotic indices are low in spite of the stimulation that colchicine is apparently capable of inducing.

The following pairs of mitotic indices were compared:

i) from roots treated with 0.025% colchicine or the mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA;

ii) from roots treated with the mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA or left to grow in the culture medium.

The difference between the former pair of mitotic indices was significant ($p = 0.001$), but the difference between the latter pair was not (Table 4). Now it has already been shown that, 24 hours after treatment ended, colchicine causes a significant increase in the MI compared with that of the control roots grown in water. Therefore, it appears that changes occur in interphase cells in roots following a colchicine treatment and these changes are reversed by IAA. Not all the concentrations of IAA in the range used in these experiments, however, produce marked reductions in the MI of the roots treated with colchicine (Table 5). The concentrations that give the most interesting results are 3-4 x 10^{-4} M IAA and, in particular 4.2×10^{-4} M IAA. At this last concentration there appears to be a balance between the stimulatory

effects of colchicine and the inhibitory effects of IAA; the result is that the MI of roots treated with 0.025% colchicine and 4.2×10^{-4} M IAA is not significantly different from that of the control roots (Table 4).

IAA also affects the percentage frequency of tetraploid cells seen in division in metaphase (Table 7). The most marked decrease in the frequency of tetraploid metaphases, occurs 24 hours after treatment with a mixture containing 3.1×10^{-4} M IAA and 0.025% colchicine. This decrease in the percentage of tetraploid metaphases accompanies the decrease in the MI. However, low frequencies of tetraploid metaphases were found in roots with mitotic indices of 4.7 and 6.0 and cannot there be due, as they could in roots with an MI of I or less, to sampling errors. There was also a decrease in the percentage frequency of tetraploid interphase cells found after colchicine and IAA treatments, in comparison to that found after treatment with colchicine alone (Table 7).

In addition to its effect on MI, IAA also affects the prophase : metaphase ratio. (It appears to have no effect on the re-establishment of normal anaphase and telophase stages, which are absent from roots even 24 hours after colchicine treatment.) The prophase : metaphase ratio in normal roots is about 3.5:I. After

colchicine treatment this ratio changes to about 0.3:1 (Table 5). A three hour treatment with colchicine and IAA does not change the prophase : metaphase ratio from the value obtained in the roots treated with colchicine alone (Table 2). After a 24 hour period of recovery following a three hour treatment with colchicine and IAA, however, the prophase : metaphase ratio is changed compared with that of the colchicine treated roots. This effect of IAA again occurs at about 4×10^{-4} M IAA (Table 5), the lower concentrations of IAA used in the mixtures with colchicine having little effect, e.g. the prophase : metaphase ratio is 0.25:1 with 0.208×10^{-4} M IAA and approximately 1:1 at 3.13 and 4.2×10^{-4} M IAA. These ratios fluctuate at higher concentrations of IAA but they are less dependable than after 4.2×10^{-4} M IAA as the MI is falling and we are dealing with relatively small numbers of cells. Thus, we see that the concentrations of IAA that reduce the MI of colchicine treated roots to that of the controls are the same concentrations that change the prophase : metaphase ratio.

The prophase : metaphase ratios from roots grown in the following solutions were compared:

i) water or a mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA;

ii) 0.025% colchicine or 4.2×10^{-4} M IAA;

iii) 0.025% colchicine or a mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA.

iv) 4.2×10^{-4} M IAA or a mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA.

With the exception of the last pair of treatments, the differences in prophase : metaphase ratios were highly significant (Table 8). IAA appears to have no effect on prophase : metaphase ratios when given alone; its effect appears to occur only in roots also treated with colchicine and then only several hours after the end of treatment. The effect of colchicine, which is to delay metaphase, is to change the prophase : metaphase ratio, compared with the control values. IAA, 4.2×10^{-4} M, in the mixtures with colchicine, changes the prophase : metaphase ratio in favour of prophases, compared with the colchicine results. This appears to be due to IAA affecting the duration of metaphase. Thus, though IAA alone does not affect mitosis in mixtures with colchicine it appears to shorten the duration of metaphase leading to a change in the prophase : metaphase ratio and a reduction in the MI.

D. General observations.

Pockets of dividing cells were observed in a predominantly non-dividing meristem, 24 hours after treatment ended,

in the squashes made from roots treated with 0.025% colchicine, and the mixtures containing 0.025% colchicine and either 3.13×10^{-4} M IAA or 4.2×10^{-4} M IAA. Such pockets of dividing cells possibly represent the initial stages of primordia formation.

The centromere gap was found to be stained in some chromosomes of the roots treated with the mixture containing 0.025% colchicine and 3.13×10^{-4} M IAA, 24 hours after treatment ended. This may indicate some effect of IAA on chromosome spiralization. Telophases with three or five groups of chromosomes were found in cells of roots treated with mixtures containing 0.025% colchicine and either 0.329 or 1.043×10^{-4} M IAA, 24 hours after treatment ended (Figures 14 and 15). This indicates that multipolar spindles or bent metaphase plates were forming, i.e. that some of the cells were beginning to recover from the effects of colchicine on spindle formation. In roots from the same fixations there were cells with micronuclei and anaphase bridges. When cells begin to recover from the effects of colchicine treatment, the anaphase separation of the chromatids is often aberrant and anaphase bridges may be found. Also some chromosomes may fail to move from the metaphase plate, resulting in the formation of micronuclei once telophase begins.

The results from this experiment suggest certain conclusions. Colchicine treatment results in an increase in M.I. Immediately after a three hour treatment with colchicine, however, the increase in MI is not significantly different from the control values. After a 24 hour period of recovery following colchicine treatment, the increase in MI is significantly different from the control values. This increase in MI is not reversed after a three hour treatment with a mixture containing colchicine and IAA; it is reversed by the higher concentrations of IAA used in the mixtures with colchicine, 24 hours after treatment ended, but not by the lower concentrations of IAA used. These observations indicate that changes occur in interphase cells in roots following colchicine treatment. These changes are reversed by high concentrations of IAA.

Also, 24 hours after colchicine treatment, the prophase : metaphase ratio has been changed compared with that of the controls due to the accumulation of metaphases. IAA, $4,2 \times 10^{-4}$ M, in the mixture with colchicine reverses much of this effect of colchicine. IAA alone, however, does not appear to affect mitosis. Therefore, IAA has an effect on mitosis in the presence of colchicine, it appears to shorten the duration of metaphase, that it does not have when used alone.

One of the consequences of colchicine treatment is that restitution occurs and, as a consequence, tetraploid cells later come into division. The percentage frequency of tetraploid metaphases and interphases induced by colchicine is reduced by the presence of IAA; compare frequencies of tetraploid cells after different treatments (Table 7). This effect increases with increasing concentration of IAA. IAA does not prevent restitution, but it appears to lengthen the mitotic cycle of tetraploid cells.

From these three results it is concluded that some of the effects of colchicine are reversible by IAA. The results indicate that colchicine may change the levels of growth factors in a root. The results of such changes are not immediately apparent, but appear 24 hours after treatment.

3. Colchicine and IAA; Changes in MI.

The results described in Section 1, Part 2, indicate that colchicine not only affects mitosis in the way that is well known, but it also has delayed effects which appear many hours later. These effects appear to be modifiable by IAA. The implications of these results are that colchicine affects mitosis by some mechanism that appears to involve changes in the level of growth

factors in roots. Because of this implication it was decided to repeat this experiment with some modifications.

Beans were grown as previously described. Some roots were treated with 0.025% colchicine, 0.329×10^{-4} M IAA, 6.26×10^{-4} M IAA, a mixture containing 0.025% colchicine and 0.329×10^{-4} M IAA or a mixture containing 0.025% colchicine and 6.26×10^{-4} M IAA; others were grown in the culture medium. Treatment was for three hours and lateral roots were fixed, either immediately or after a 24 hour recovery period following treatment. Permanent squash preparations were made and, as in the previous experiment, 6,000 cells were scored for each treatment.

1. Treatment for three hours.

Roots treated with 0.025% colchicine have more than double the MI of the control roots. Roots treated with either of the concentrations of IAA used have similar mitotic indices to the control roots. However, mixtures containing 0.025% colchicine and IAA, at a concentration of either 0.329 or 6.26×10^{-4} M have mitotic indices above the control value (Table 9; Figure 5).

The difference between the mitotic indices of roots from the following pairs of treatments were tested statistically:

i) control or 0.025% colchicine;

ii) control or the mixture containing 0.025% colchicine and 0.329×10^{-4} M IAA;

iii) control or the mixture containing 0.025% colchicine and 6.26×10^{-4} M IAA;

iv) 0.025% colchicine or the mixture containing 0.025% colchicine and 6.26×10^{-4} M IAA;

v) 0.025% colchicine or the mixture containing 0.025% colchicine and 0.329×10^{-4} M IAA;

vi) control or 0.329×10^{-4} M IAA;

vii) control or 6.26×10^{-4} M IAA;

viii) 0.025% colchicine or 6.26×10^{-4} M IAA;

ix) 0.025% colchicine or 0.329×10^{-4} M IAA.

These differences were significant in (i), (iv), (viii) and (ix) and not significant in (ii), (iii), (v), (vi) and (vii) (Table 11).

A number of conclusions can be drawn from these results. First, within a three hour treatment, 0.025% colchicine has increased the MI significantly compared with the control values. In the previous experiment, colchicine treatment resulted in an increased MI, but not a significant increase. Therefore, the increase in MI found after a three hour treatment with colchicine may, or may not, be statistically significant, depending on the population of beans examined. Secondly, whereas treatment

with colchicine results in an increase in MI, treatment with IAA does not change the MI significantly, whether colchicine is present or not. This was also found in the previous experiment. These results indicate that IAA has, within a three hour period of treatment, reversed most of the colchicine induced increase in MI. However, whereas there is no significant difference between the mitotic indices of the roots treated with colchicine alone or those treated with colchicine and 0.329×10^{-4} M IAA, there is a significant difference between the mitotic indices of the roots treated with colchicine or those treated with the mixture containing colchicine and 6.26×10^{-4} M IAA. These results indicate that though IAA reverses most of the colchicine induced increase in MI, this reversion is not complete except with the higher concentration of IAA used. Thus colchicine and IAA have different effects on MI.

IAA, in either of the concentrations used, has had no marked effect on the percentage of cells in the various mitotic phases (Table 9). This was also found to be the case in the previous experiment. Within a three hour period, IAA does not affect mitosis and neither colchicine nor the mixtures of colchicine and IAA, in this or the previous experiment, have markedly altered the numbers of cells in prophase (Tables 2 and 9). This suggests that after a three hour treatment, none of the colchicine

treatments have affected the entry of cells into mitosis, whether IAA was also present or not. Treatment with colchicine or the mixtures containing colchicine and IAA, leads to an increase in the number and percentage of cells in metaphase (Table 9; Figure 6). Similar results were also obtained in the previous experiment (Tables 2 and 3; Figure 2). The difference between the number of cells in metaphase in the controls and in the roots treated with colchicine was found to be highly significant ($p = < 0.001$). The increase in metaphases in the colchicine treated roots is due both to the prevention of the anaphase separation of the chromatids and to a delay induced at metaphase (Table 9). These effects of colchicine are not reversed by IAA within a three hour period.

A consequence of the delay induced by colchicine at metaphase is that the prophase : metaphase ratio is changed. The difference between the prophase : metaphase ratios after the following treatments was tested statistically:

i) control or 0.025% colchicine;

ii) control or the mixture containing 0.025% colchicine and 0.329×10^{-4} M IAA;

iii) control or the mixture containing 0.025% colchicine and 6.26×10^{-4} M IAA;

iv) 0.025% colchicine or the mixture containing 0.025% colchicine and 6.26×10^{-4} M IAA;

v) 0.025% colchicine or the mixture containing 0.025% colchicine and 0.329×10^{-4} M IAA;

vi) 0.329×10^{-4} M IAA and the mixture containing 0.025% colchicine and 0.329×10^{-4} M IAA;

vii) 6.26×10^{-4} M IAA and the mixture containing 0.025% colchicine and 6.26×10^{-4} M IAA.

With the exception of (iv) and (v), all of these differences were significant (Table 12).

Two conclusions can be drawn from these results. First, within a three hour treatment, colchicine has changed the prophase : metaphase ratio, because of the delay it induces at metaphase. Secondly, this change is not prevented by IAA within a three hour treatment, i.e. IAA in mixtures with colchicine does not affect the duration of metaphase within a three hour treatment.

2. Treatment for three hours and 24 hours recovery.

Roots were treated with 0.025% colchicine and were examined 24 hours after treatment ended. These roots had a MI about twice that of the controls. IAA, in both of the concentrations used has reduced the MI below the value found in the controls. In the presence of colchicine, both concentrations of IAA reduce the MI, compared with that of the colchicine treated roots (Table 10; Figure 7).

The difference between the mitotic indices of roots after the following treatments was tested statistically:

i) control or 0.025% colchicine;

ii) control or the mixture containing 0.025% colchicine and 0.329×10^{-4} M IAA;

iii) control or the mixture containing 0.025% colchicine and 0.025% colchicine and 6.26×10^{-4} M IAA;

iv) 0.025% colchicine or the mixture containing 0.025% colchicine and 6.26×10^{-4} M IAA;

v) 0.025% colchicine or the mixture containing 0.025% colchicine and 0.329×10^{-4} M IAA;

vi) 0.025% colchicine or 6.26×10^{-4} M IAA;

vii) 0.025% colchicine or 0.329×10^{-4} M IAA;

viii) control or 6.26×10^{-4} M IAA;

ix) control or 0.329×10^{-4} M IAA.

These differences were significant in (i), (iv), (vi), (vii) and (viii), and were not significant in (ii), (iii) and (ix) (Table 11).

We see several things from these results. First, mitotic index is significantly reduced by the higher concentration of IAA but not the lower concentration of IAA. However, immediately after a three hour treatment neither of these concentrations of IAA affects the MI (Tables 2 and 9; Figures 1 and 5). Thus, IAA affects MI only in the higher concentrations used and then only several hours after treatment (Tables 5 and 10; Figures 3 and 7). Secondly, 24 hours after treatment with 0.025% colchicine the MI is significantly higher than the control values. This

confirms the result of the previous experiment. Therefore, not only is there an increase in MI immediately after colchicine treatment, but this increase is maintained even 24 hours after treatment. Thirdly, 0.329×10^{-4} M IAA in a mixture with colchicine changes the MI, but we see that the increase in MI as compared with the controls is not significant nor is the decrease as compared with roots treated only with colchicine. Thus, the effect of this concentration of IAA partially offsets the change induced by colchicine whereas 6.26×10^{-4} M IAA mixed with colchicine completely prevents the increase in MI and even reduces it to values lower than the controls, i.e. 3.30 compared with 6.03 (Table 10). Though these two values, 3.30 and 6.03 are not significantly different (Table 11), 1.62 and 6.03, i.e. values for roots treated with IAA alone and the controls, are significantly different. We can conclude, therefore, that colchicine has mitigated the inhibitory effects of 6.26×10^{-4} M IAA on the mitotic cycle.

The comparison of mitotic indices from roots fixed 24 hours after the end of treatment indicates clearly that colchicine or IAA alone induce significant changes in the MI; colchicine increases and IAA decreases the MI. The weaker concentration of IAA used in this experiment does not reduce the MI significantly

either when given alone, cf. 5.40 and 6.03, or when given with colchicine, c.f. 9.18 and 12.33. However the stronger concentration of IAA does lower the MI significantly; when given alone the MI is reduced to 1.62 as compared with 6.03 and when given with colchicine the MI is reduced from 12.33 to 3.30 (Tables 10 and 11; Figure 7). These results confirm those obtained in the previous experiment (Table 5; Figure 3). Both sets of results indicate that the weak solutions of IAA used in these experiments modify the effects of colchicine but do not completely reverse them, while the strongest solution of IAA used reduces the MI significantly and appears to prevent the stimulation of the mitotic cycle that follows treatment with colchicine. These results can also be interpreted from the point of view of the effect of IAA; then it would be said that while the weak solution of IAA does not reduce MI significantly, the strong solution does so and this inhibition of the mitotic cycle can be reversed by simultaneous treatment with 0.025% colchicine (cf. Tables 5 and 10). These results suggest that colchicine treatment results in a lowering, in the root apex, of the concentration of growth factors that control mitotic cycles and that there is a partial restoration of these levels with 0.329×10^{-4} M IAA and overcompensation with 6.26×10^{-4} M IAA.

IAA has little effect on the percentages of cells in the various mitotic phases, 24 hours after treatment ended (Table 10). This was also found in the previous experiment (Table 5). Colchicine and the mixtures containing colchicine and IAA have, on the otherhand, maintained a higher percentage of metaphases than the controls, as was found in the last experiment (Tables 6 and 10). The difference between the number of metaphases in the control or the colchicine treated roots was found to be highly significant ($p = <0.001$). Thus the increased number of cells in metaphase found after a three hour treatment is maintained even 24 hours after treatment. This result, along with that obtained in the last experiment indicates that the colchicine induced delay in metaphase persists many hours after treatment (Figure 8).

The difference in the prophase : metaphase ratios between the following treatments was tested statistically:

i) control or 0.025% colchicine;

ii) control or the mixture containing 0.025% colchicine and 0.329×10^{-4} M IAA;

iii) control or the mixture containing 0.025% colchicine and 6.26×10^{-4} M IAA;

iv) control or 0.329×10^{-4} M IAA;

v) control or 6.26×10^{-4} M IAA.

With the exception of (iv) and (v), these differences were significant (Table 12).

It appears that IAA, even 24 hours after treatment, has not changed the prophase : metaphase ratio significantly from the control values. This result, the results obtained for anaphase and telophase (Table 10) and the results obtained in the previous experiment, all suggest that IAA does not affect mitosis. Following colchicine treatment, the prophase : metaphase ratio is lower than that of the controls, due to the fact that colchicine lengthens the duration of metaphase. Neither of the concentrations of IAA used changed, to a significant degree, the effect of colchicine in lowering the prophase : metaphase ratio. In the previous experiment, the higher concentrations of IAA in the mixtures with colchicine changed the prophase : metaphase ratio significantly from values found for roots treated only with colchicine (Table 8). This effect has been found in the present experiment though not to such a marked extent (Table 10).

From the two experiments certain important results are obvious. They are:

i) colchicine increases the MI due partly to metaphase delay and partly to a stimulation of the mitotic cycle.

ii) IAA in concentrations of up to 4.2×10^{-4} M reduces the MI apparently by reducing the number of metaphases,

i.e. the IAA reverses the colchicine induced metaphase delay. This is seen in the changes in prophase : metaphase ratios;

iii) stronger concentrations of IAA also reduce the MI, but they do so by lowering the actual number of cells in prophase;

iv) colchicine can prevent the inhibitory effects of IAA but only up to 4.2×10^{-4} M; it does not prevent the inhibition induced by stronger concentrations.

These results suggest that mitotic cycles are affected in the period following treatment with colchicine. IAA and colchicine produce opposite effects in this period, suggesting that one of the consequences of a colchicine treatment may be a change in the level of IAA in the root (see Discussion pp. 53-66).

4. Colchicine and IAA; Changes in MI.

The suggestion that changes occur in the level of growth factors in roots that have been treated with colchicine has implications, for cell division and root growth, that extend beyond a 24 hour period. The treatment of roots with colchicine and, or, IAA as described previously has been extended and modified in the following experiment. The response of treated meristems has been followed over several days in order to determine the long term changes that occur in meristems that show an initial stimulation or inhibition of mitosis, depending upon the treatment used.

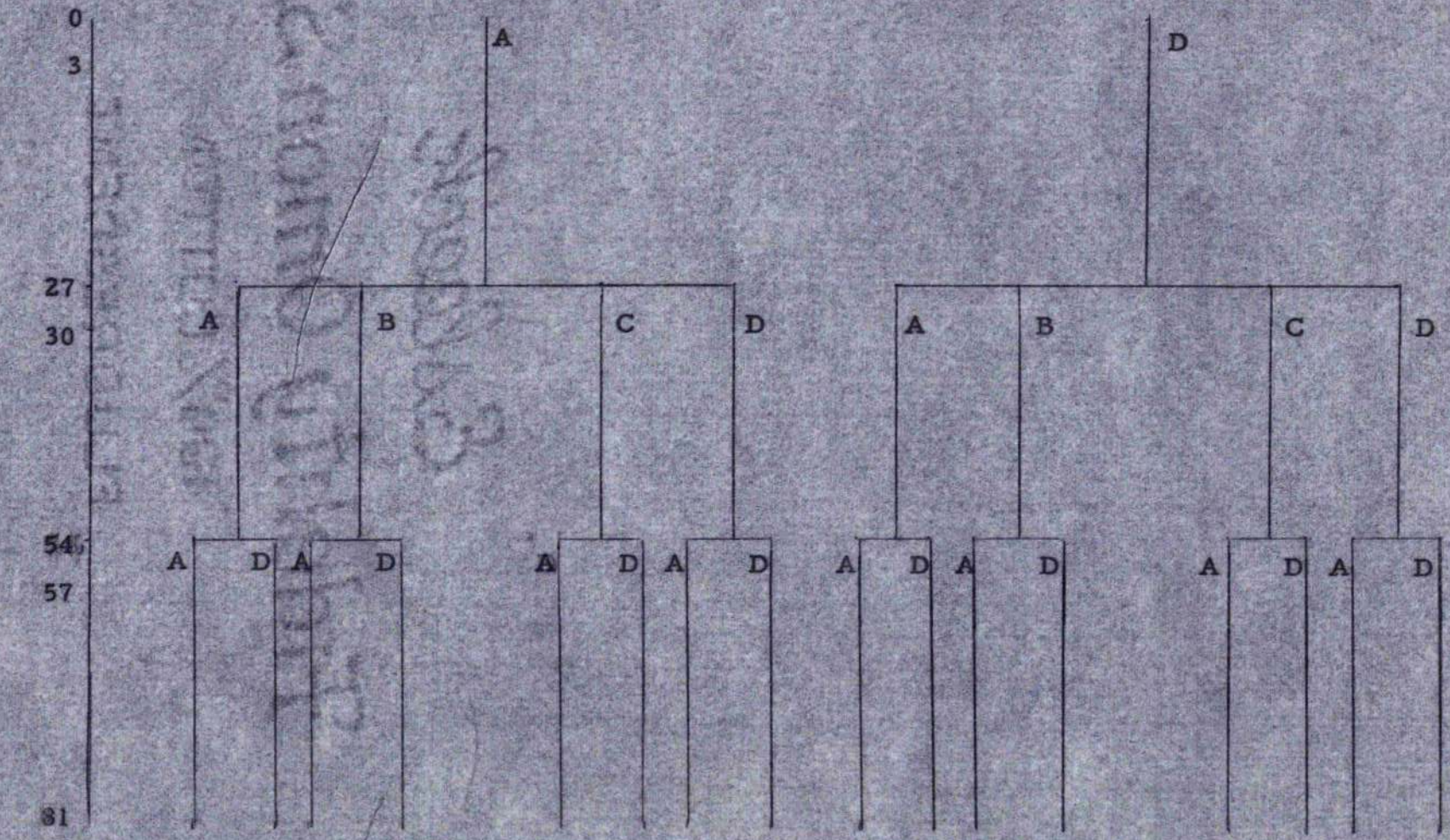
Beans were grown as before. Roots were treated with 4.7×10^{-4} M IAA and, or 0.025% colchicine according to the scheme laid out in Figure 9. One day intervals separated each treatment. Treatment was for three hours in each case. Lateral roots were fixed immediately before the first, second and third treatments began, immediately after the third treatment ended, and every 24 hours thereafter until six days after the third treatment ended. As before, ten permanent squash preparations were made and 6,000 cells scored for each treatment.

A. IAA.

1. MI in roots 24 hours after treatment.

Roots were treated at 0-3, 27-30 and 54-57 hours after the experiment began and fixed 24 hours later, i.e. at 27, 54, 57 and 81 hours after the experiment began. Mitotic index fell in all treated roots (Tables 13a-h; Figure 10). Roots which were treated with IAA at the beginning of the experiment, that is roots from beans fixed 216 hours from the beginning of soaking died whether they were subsequently treated with IAA or colchicine. The data from these beans has been included in the tables but no comparisons have been made with data from other treatments in case changes in these roots could be due to incipient death.

Figure 9



A = a 4.7×10^{-4} M solution of IAA; B = a 0.025% solution of colchicine;

C = a solution containing 4.7×10^{-4} M IAA and 0.025% colchicine; D = controls, grown in the culture medium.

The differences in MI after the following treatments were examined:

i) control or roots treated with 4.7×10^{-4} M IAA 27 hours after the experiment began and fixed 54 hours after the start of the experiment;

ii) control or roots treated with 4.7×10^{-4} M IAA 54 hours after the experiment began and fixed 81 hours after the start of the experiment.

These differences were found to be significant (Table 14). Therefore IAA reduces the MI of the root apex within 24 hours of treatment ending, regardless of the age of the roots at the time of treatment.

2. MI : recovery from IAA induced inhibition.

Roots treated with IAA 27 and, or, 54 hours after the experiment began , recover from the effects of the treatment in the following three to five days and their mitotic indices return to normal. For example, roots treated at 27 hours show a low MI for the following two days (Table 13b); the MI falls to 2 or less and does not reach values similar to those of the controls for three to four days. A similar response is shown by roots treated 54 hours after the beginning of the experiment. These roots, however, recover faster than roots treated at 27 hours (cf. Tables 13b and 13c). Roots treated 27 and 54 hours after the experiment began,

recover at a slower rate than those treated at only one of these times (Table 13d). This suggests that the age of the roots at the time of treatment is an important factor in determining their response to IAA, at least as seen in changes in MI.

These changes in MI are due to a reduction in the numbers of cells in the various mitotic stages in the treated roots (Tables 13a-d). There is no marked change in the percentages of cells in the different stages of mitosis except in those roots in which the MI is 0.5 or less (Table 16) and in those cases the low numbers of cells seen in division make comparisons less valid than when we are dealing with high mitotic indices and large numbers of cells. In contrast with roots treated with colchicine (Section 1, Parts 2 and 3), there is little change in prophase : metaphase ratios except in the cases of roots with very low mitotic indices. Thus it appears that IAA does not affect the percentages of cells in the various stages of mitosis though apparently it does lengthen the mitotic cycle or prevents the entry of cells into prophase.

B. Colchicine.

Only a single treatment with colchicine was given and the response of the roots was followed over seven days. The increase in MI 24 hours after treatment that has been described previously

was again observed; MI increased to 19.0 (cf. control value of 5.28). The difference between these two values is highly significant (Table 14). After this initial rise, however, the MI begins to fall (Table 13i; Figure 11). By two and three days after treatment it has fallen to 4.0 and 0.9 respectively (cf. control values of 6.9 and 5.0). In the following 4 days MI in these roots remains low (Table 13i). Thus the initial increase in MI after colchicine treatment is followed by a rapid decrease until it is 1.0 or less.

Colchicine has little effect on the number of cells in prophase, 24 hours after the end of treatment, indicating that the rise in MI found 24 hours after colchicine treatment ended is a result of metaphase delay and not to an increase in the number of cells in prophase. The number of metaphases is more than twenty-one times greater than the control value (Tables 13a and 13i; Figure 12). This increase in the number of metaphases is highly significant ($p = < 0.001$). Within a further three hours, however, the number of cells in metaphase has decreased to about six times the control figure and it continues to decrease throughout the duration of the experiment (Table 13i).

As previously found, roots treated with colchicine also show a change in the ratio of prophases : metaphases (Table 16). The

change in the prophase : metaphase ratio is significant in 24 hour fixations (Table 15). In subsequent fixations the prophase : metaphase ratios also differed from the controls but the differences have not been tested statistically because mitotic indices were low and the numbers of cells available for comparison were small (Tables 13i and 16).

Cells are seen in division for a period of several days after colchicine treatment but the numbers are low 48 hours or more after treatment. Thus the initial increase in MI does not persist and is followed by a decrease which continues for the duration of the experiment. The increase in MI 24 hours after treatment and the decrease in MI 153 hours after treatment are both significant (Table 14; Figure 11). These results again suggest a change in the level of growth factors in treated roots; the fall in MI that follows the initial increase may indicate that the level of growth factors in the roots reaches levels that will not support normal mitotic activity. Results from experiments that are reported below (this section, Part 4D) suggest that the eventual fall in MI in colchicine treated roots is due to low levels of growth factors since there is partial recovery of the MI following treatment with IAA (Figure 11).

C. Colchicine and IAA : simultaneous treatment.

It was again found that IAA prevents the increase in MI found 24 hours after treatment with colchicine (Table 13j). The subsequent behaviour of the roots is similar to that found after either colchicine or IAA, i.e. the MI remains low, 2.1 or less, for 7 days. The low MI found 24 hours after treatment with IAA and colchicine is lower than values previously found after treatment with solutions of the same strengths, but the trend is the same. The difference in the response of the roots at different times suggests slightly different degrees of sensitivity of different batches of beans. Differences between mitotic indices of roots from various treatments were again compared:

i) control or the mixture containing 0.025% colchicine and 4.7×10^{-4} M IAA;

ii) 4.7×10^{-4} M IAA or the mixture containing 4.7×10^{-4} M IAA and 0.025% colchicine;

iii) 0.025% colchicine or the mixture containing 0.025% colchicine and 4.7×10^{-4} M IAA.

With the exception of (ii) the differences were significant (Table 14). Again we see that IAA can reverse the mitotic stimulation induced by colchicine and in the present experiment treatment with 4.7×10^{-4} M IAA has reduced the MI to a value that is just significantly lower ($p = 0.02 - 0.01$) than that of the controls.

Other roots which had been treated with a mixture of colchicine and IAA 24-27 hours after the experiment began were also treated, 24 hours later, with IAA (4.7×10^{-4} M). Mitotic indices were determined for the following 6 days and, as in roots treated with a mixture, they remained low (Table 13k).

Roots that were treated at the beginning of the experiment with IAA were found to die whether or not they were subsequently given other treatments. It appears that colchicine does not modify the initial lethal response to IAA (Tables 13l and m).

In the period in which the mitotic indices of treated roots are low, the numbers of cells in the various stages of mitosis are also low and they show considerable variation from day to day (Tables 13j and k). Prophase : metaphase ratios, therefore, also vary, though for the most part they are of little value because they are based on very small numbers of cells. Prophase : metaphase ratios have been compared, however, in roots with high mitotic indices in order to determine whether the results from the present and the previous experiments are similar. The following were examined 24 hours after treatment ended.

i) control or the mixture containing 0.025% colchicine and 4.7×10^{-4} M IAA;

ii) 4.7×10^{-4} M IAA or the mixture containing 0.025% colchicine and 4.7×10^{-4} M IAA;

iii) 0.025% colchicine or the mixture containing 0.025% colchicine and 4.7×10^{-4} M IAA;

iv) 4.7×10^{-4} M IAA or 0.025% colchicine.

These differences were not significant in (i) and (ii) but were significant in (iii) and (iv) (Table 15).

Though IAA itself has, unlike colchicine, little or no effect on the prophase : metaphase ratio, it reverses the effect of colchicine in producing prophase : metaphase ratios different from those of control roots. IAA does not, however, prevent the inhibitory effect of colchicine on anaphase. The reappearance of anaphase and telophase figures did not take place any faster in roots treated with both IAA and colchicine than in the roots treated only with colchicine (Tables 13i, j, and k). Therefore, the time at which spindles reappear after colchicine treatment is not influenced by IAA and it appears that IAA is not a factor involved in spindle formation.

D. Colchicine followed by IAA.

Roots were treated with colchicine at 24-27 hours after the beginning of the experiment and then treated with IAA 24 hours later. The fall in MI in the following 27 hours is greater than in roots treated only with colchicine (Tables 13i and n; Figure 11); thus the MI is 4.0 two days after treatment with colchicine and

0.7 at the same time if treatment with IAA occurred one day after colchicine. Though the initial drop is greater in roots given IAA one day after colchicine, the mitotic index recovers faster. Thus the difference between mitotic indices in roots treated with colchicine and colchicine followed by IAA is significant 177 hours after the beginning of the experiment (Table 14). The MI from the latter treatment was also compared with controls and the difference between them was not significant (Figure 11, day 7) 153 hours after the beginning of the experiment.

Following treatment with colchicine and then 24 hours later with IAA, prophase : metaphase ratios differ from those of roots treated only with colchicine. In one respect this agrees with previous observations. However, the times at which the prophase : metaphase ratios change differ in the present experiment from those reported in earlier sections. The first difference is seen (Table 13n) in the time at which normal prophase : metaphase ratios are seen, i.e. they are found 24 hours earlier than in roots treated only with colchicine (Table 16). The second difference in the response of roots to colchicine followed by IAA is seen at 57 hours. A comparison of the numbers of cells in prophase and metaphase at the beginning and at the end of a three hour treatment with IAA (Table 13n, 54 and 57 hours) shows that

there is no change in the number of prophases but there is a significant fall in the number of metaphases, i.e. from 979 to 466 (Figure 12). It appears that simultaneous treatment with IAA and colchicine does not produce a detectable effect on the duration of metaphases after three hours; however, if the IAA treatment is given 24 hours after the colchicine, i.e. at the time when the MI is at a maximum, then IAA will in a three hour treatment reduce the number of metaphases.

E. Frequency of polyploid cells in division.

No polyploid cells were present in the control roots, or, in any of the roots treated with a 4.7×10^{-4} M solution of IAA. IAA, in the concentration used, does not induce polyploidy.

It was found that, 24 hours after colchicine treatment ended, about 15% of the metaphases were polyploid (Table 17). This increases to about 50% within a further 27 hours, after which the percentage of polyploid metaphases decreases. In roots treated with colchicine and subsequently with IAA, the percentage of polyploid metaphases found was never higher than 18% of all metaphases (Table 18) and had fallen to 2% by the end of the experiment. Treatment with IAA appears to reduce the number of polyploid cells seen in division for there were always fewer

polyploid cells in roots that were treated with colchicine and then IAA than in roots treated only with colchicine (Tables 18 and 19).

It is interesting that roots treated with a mixture of colchicine and IAA show low frequencies of polyploid metaphases within the first two days of treatment, but eventually show an increase (Table 20). The presence of 25% polyploid metaphases six days after treatment with IAA and colchicine suggests that the polyploid cells induced by colchicine are not incapable of division but that the duration of their mitotic cycle has been extended due to the treatment with IAA.

5. IAA effects during interphase.

All the evidence from the previous experiments indicates that IAA has no effect on mitosis at the end of a three hour treatment. The results reported in the previous parts of this section show that even the strongest concentration of IAA used, 6.26×10^{-4} M, does not produce significant changes in the MI of treated roots nor in the percentages of cells in the various stages of mitosis (Tables 2, 3, 13b, c and d). However, sometime after the end of a three hour treatment the MI of treated roots falls (Tables 5, 6, 13b, c and d) and the decrease is significant, at least after the highest concentrations used. At the same time there is no change in the percentage of cells in the various mitotic stages. These data and

those on the frequencies of tetraploid metaphases seen after treatment with colchicine and IAA suggest that IAA has an effect sometime during interphase. The effect appears to induce an increase in the duration of the mitotic cycle. This possibility has been investigated in the present experiment.

Beans were grown in the way previously described. Roots were treated with a 6.26×10^{-4} M solution of IAA for three hours. Lateral roots were fixed in acetic-alcohol 3, 8, 14 and 26 hours after treatment began. 20 permanent squash preparations were made for each fixation and 1,000 cells were scored from each slide, giving a total of 20,000 cells scored for each treatment. Controls were also scored at these times.

It can be seen that this treatment has had little effect on the percentages of cells in the different stages of mitosis. At all four fixation times these percentages are similar to those of controls (Tables 21a and b). Anaphase figures were absent 14 hours after treatment began (Table 21b), but this is a sampling error due to the low numbers of cells in division; mitotic index at this time was 0.45.

Eight hours after the beginning of treatment a fall in MI is detectable, the MI, 3.4, is half that in the controls, where the MI is 6.9. After a further six hours, the MI in treated roots

has fallen to less than 1 and it remained at this level in the subsequent fixation.

Mitotic indices of the controls and the treated roots have been compared. At three hours the difference between treated roots and controls was not significant. By eight hours the difference is on the borderline of statistically acceptable significance, $p = 0.05 - 0.02$; this indicates that by eight hours the MI is beginning to fall though it is not yet highly significant. At 14 and 24 hours, however, $p = < 0.01$ and the differences are highly significant. Thus the trend in the fall in MI that is first seen at eight hours continues in the following 18 hours.

These results indicate that IAA inhibits some interphase process and this results in a fall in MI. Since the decrease in MI is first seen five hours after the end of treatment, the inhibition appears to begin to have an effect even in G_2 though the maximum effect of IAA treatment is not seen till later, after a period in which it could have affected G_1 or S.

Section 1 : Summary.

The results obtained in the previous experiment are as follows:

i) IAA, in the concentrations used, does not affect the percentages of cells in the various stages of mitosis. The highest concentration used, however, causes a reduction in MI within five hours of treatment ending;

ii) the colchicine induced inhibition of anaphase is not reversed by IAA and is not a pH effect;

iii) following colchicine treatment the number of metaphases increases. This results in a change in the prophase : metaphase ratio. This change in prophase : metaphase ratio is reversed by the higher concentrations of IAA used, but only many hours after treatment;

iv) restitution occurs after colchicine treatment and as a consequence polyploid cells are seen in division one mitotic cycle later. Treatment with mixtures of colchicine and IAA, or with IAA after colchicine treatment, leads to a reduction in the frequency of polyploid metaphases;

v) there is an increase in MI within 24 hours of treatment with colchicine. After a further 24 hours the MI decreases and remains low for the following five days. The initial increase in MI is prevented by simultaneous treatment with the higher concentrations of IAA used in the mixtures with colchicine.

It is suggested that, following colchicine treatment, there is a decrease in the level of growth factors in the root apex. This decrease can be partly compensated for by the addition of IAA.

Discussion

A. Colchicine

The immediate effect of colchicine on mitosis is to prevent the anaphase separation of the chromatids (Levan, 1938). This effect can be detected within five minutes of the beginning of treatment with 5×10^{-7} M colchicine (Taylor, 1965). Colchicine, however, also has effects on mitosis which normally require a longer period of treatment: these are;

(i) an increase in MI

(ii) an increase in the numbers of cells in metaphase

(iii) a change in the prophase:metaphase ratio

(iv) polyploid cells come into division

In the experiments described in this section, the MI increased at the end of a three hour treatment. An increase in the MI has been reported frequently (see references in Eigsti and Dustin, 1955) and is due to the accumulation of cells in metaphase. The increase in MI within a three hour period may or may not be significant depending on the population of beans used, but it is highly significant after a 24 hour recovery period i. e. MI continues to show an increase even 24 hours after the end of treatment. Evans, Neary and Tonkinson (1957) also found such an effect, but they treated with colchicine continuously and not, as in the experiments reported here, for only three hours. These results are in contrast to those obtained by Van't Hof and Wilson

(1962) who found that treatment of Pisum for 30 minutes with 3.76 x 10^{-4}M colchicine resulted in no accumulation of c-metaphases and no increased MI. It may be that Pisum responds differently to colchicine treatments with respect to changes in MI but it is clear that this is not the result of insensitivity to colchicine since polyploidy is induced in Pisum by colchicine as Van't Hof and Wilson (1962) found in their experiments.

A significant increase in the number of metaphases was also found in the experiments described in this section, 24 hours after treatment ended. Such increases have been reported previously (Evans, Neary and Tonkinson, 1957) but only with continuous treatments. The increase in the number of metaphases contributes to the increase in MI found at all times up to 24 hours after treatment, but not all increases in MI are due solely to metaphase delay. Evidence has been presented here (Section 1) that a mitotic stimulation occurs some time after treatment with colchicine. It is clear that all metaphases are not held at this stage indefinitely; restitution nuclei can be seen as early as three hours from the beginning of treatment, showing that at least in some cells, regression to the interphase condition occurs quickly. Furthermore, if cells were held at metaphase for many hours then mitotic indices would rapidly exceed the levels that have been found, since if all metaphases undergo arrest and entry into prophase is not inhibited, then the MI

would be expected to double about every four hours, which is the length of mitosis in V. faba roots (Howard and Pelc, 1953). In fact maximum reported values do not exceed 34.9 (Evans, Neary and Tonkinson, 1957). The second main line of evidence that arrested cells undergo restitution and pass into interphase is that these cells appear 24 hours later as tetraploid cells. Not only do tetraploid cells appear in division one day after colchicine treatment but they may occur with a high frequency (Van't Hof and Wilson, 1962; Murín, 1964; Davidson, 1965; Davidson, MacLeod and O'Riordan, 1965). It has been pointed out that tetraploid metaphases make a significant contribution to mitotic indices 24 hours after treatment with colchicine and, even if allowance is made for the fact that they too show metaphase delay, it is clear that without the tetraploid metaphases the total number of cells in division, the MI, would be significantly lower (Davidson, MacLeod and O'Riordan, 1965). Tetraploid cells coming into division after treatment with colchicine may show some synchrony. Thus, failure to catch the peak of synchronised cells would lead to lower frequencies of tetraploid cells appearing in some experiments as compared with others. It appears that, in the roots described in Section 1, the fixations did not coincide with the peak of synchrony, for the values of tetraploid metaphases, 15-47%, are lower than those reported previously (Van't Hof and Wilson, 1962; Murín, 1964; Van't Hof, 1965; Davidson, 1965).

However, even with these values, the tetraploid metaphases make a significant contribution to the MI and without them the mitotic indices would approximate more closely to the control values. This evidence suggests that there is a stimulation of mitotic activity in the 24 hour period following treatment with colchicine.

In roots fixed more than 24 hours after colchicine treatment, the MI is found to fall until, three days after treatment, it is less than 1. Mitotic indices remain low, 1 or less, for the following four days (section 1; Davidson, MacLeod and O'Riordan, 1965). If metaphase delay was solely responsible for changes in MI within 24 hours of treatment with colchicine, then we would expect the MI to return to normal once normal spindles begin to form again. However, anaphase figures were seen within two days of treatment ending at the same time as the MI was falling to a value below that of the controls. The re-establishment of normal division and the disappearance of metaphase delay (Table 13i) does not result in normal mitotic indices; normal or nearly normal levels of mitotic activity are not established until several days after metaphase delay has disappeared.

One of the conclusions drawn from the results of treatments with colchicine and IAA is that a change in the level of growth factors occurs in roots treated with colchicine and that this is reversed by IAA. Though it appears that a change in the levels of growth factors does occur in treated roots, it seems that it is not simply a suppression of synthesis

of an IAA-like auxin. The results reported here show that treatment with IAA after colchicine does not induce an immediate return to normal levels of mitotic activity i. e. the MI in roots given such double treatments does not become normal at once, though it does recover faster than in roots that do not receive the IAA treatment. Comparable results have been reported from studies on root growth where it was shown that IAA treatments given one day after colchicine stimulated the growth of the roots (Davidson, MacLeod and Taylor, 1965). Roots given IAA after colchicine produce significantly greater growth than roots given only colchicine. This stimulation does not become visible immediately however, for the IAA treatment did not induce faster recovery from the effects of colchicine; its affect was to induce faster growth once regeneration had begun.

Other evidence also indicates that the changes that occur in colchicine treated roots are complex. The induction of polyploidy in meristems is associated with an eventual fall in MI (Section 1; Davidson, MacLeod and O'Riordan, 1965) but is not always associated with an increase in the duration of the mitotic cycle. Induced polyploidy has been used as a convenient marker for estimating the duration of mitotic cycles in treated roots. Van't Hof (1965) has shown that the duration of the mitotic cycle of polyploid cells induced by colchicine shows little change over three successive divisions. In primordia of V. faba roots, octoploid cells appear within 48 hours of colchicine treatment, indicating

three successive divisions in 48 hours and providing no evidence that the duration of the mitotic cycle had increased following treatment with colchicine (Davidson, 1965). Following irradiation it has been shown that some cells, carrying chromosome changes that act as cell markers, divide once every 24 hours over a seven day period and that in that period there is little or no root growth and low mitotic acitivity (Davidson, 1965). All these results indicate that in a meristem that has been disrupted by treatment, some cells maintain normal mitotic activity even though the average activity of the general meristem is low. It is in this period that cells in the quiescent centre come into division and begin to contribute to meristem growth (Clowes, 1954).

The conclusion that can be drawn from these results is that though disrupted roots do not support normal levels of mitotic activity and are growing slowly or not at all, they will support active division in a limited number of cells. This mitotic activity has been detected by the use of marked cells and it has been shown by following the composition of regenerated meristems that the cells that remain capable of division contribute to the formation of new meristems; i. e. the decendents of these cells occur in regenerated meristems (Brumfield, 1943; Davidson, 1960;1961). The factors controlling this mitotic activity in a limited population of cells is obviously a crucial factor in root regeneration (Clowes, 1961) but the mechanism underlying it is completely unknown.

B. IAA

IAA has no detectable effect on the percentages of cells in the various stages of mitosis (Section 1; Chouinard, 1955). Though there is no effect on MI within three hours, a fall in MI is found 24 hours later. This could be due to a speeding up of mitosis or to an increase in intermitotic times. Chouinard (1955) working with Allium roots and with concentrations of IAA (3-27 ppm) that are weaker than those used in the present experiments, reports an increase in MI.

IAA can stimulate mitosis in non-dividing cells e. g. pericycle cells (Goldacre, 1959) and mature cells of roots (Levan, 1939; D'Amato, 1945) and it is necessary for DNA doubling and mitosis in tobacco tissue explants (Patau, Das and Skoog, 1957). It is also necessary for cell division in tomato crown gall tumour callus cultures (Klein and Vogel, 1956). Naylor (1958), however, found that NAA, a similar compound to IAA, prevented the replication of DNA in lateral bud promeristem nuclei after the removal of the terminal bud. Thus, though IAA is a necessary factor for mitosis in vivo and DNA synthesis in vitro, Naylor's results show that an auxin is responsible for the in vivo suppression of DNA synthesis. This suppression would lead to a prolongation of interphase and to a long intermitotic time. An auxin can act as an inhibitor and prolong mitotic cycles.

Cell elongation is also affected by IAA. Roots grown in vitro appear to require IAA for cell elongation (Bonner and Koepfli, 1939)

Lundegårdh, 1942; Burström, 1942; Street et al, 1954). Pilet (1951) has suggested that the highest concentration of IAA in the root occurs in the apical meristem and it is interesting that while low doses of X-rays will inhibit root growth if delivered to the apical meristem, massive doses are needed to inhibit root growth by direct irradiation of the zone of elongation (Gray and Scholes, 1951; Gray and Boag, unpublished, quoted by Howard and Pelc, 1953). These results should be related to the observation that IAA biosynthesis is very sensitive to X-rays (Gordon, 1956). On the basis of all these results it has been suggested that IAA synthesis occurs in the root apical meristem (Davidson, 1960; Torrey, 1963) and that one of the events in the recovery of roots from irradiation is the re-establishment of auxin synthesis (Davidson, 1961). That suggestion is obviously compatible with the result that IAA given one day after treatment with colchicine stimulates root growth (Davidson, MacLeod and Taylor, 1965).

Roots of V. faba have a quiescent centre in which cells rarely synthesize DNA and seldom divide (Clowes, 1959). When roots are irradiated (360 r), many of the cells of the meristem stop dividing and the cells of the quiescent centre synthesize DNA, enter division and contribute to the formation of a new meristem (Clowes, 1959, 1961). This mitotic activity in a small group of cells of irradiated roots may be analogous to the normal mitotic activity that has been described in some cells in roots treated with colchicine or X-rays (see previous

section of Discussion). The question that relates to those cells also relates to cells of the quiescent centre, that is, what factors are present in these treated roots that support division in a limited number of cells but not in the whole meristem? Furthermore, we must ask, with specific relevance to the behaviour of the quiescent centre cells, what changes in the levels of growth factors must occur in order to stimulate division in cells that do not normally divide while inhibiting division in cells that do normally divide? Though direct experimental evidence is lacking, it is possible that the region of the quiescent centre coincides with the highest concentration of IAA in the meristem and that the presence of the former depends upon the latter.

One line of evidence supports the suggestion that high concentrations of IAA can keep cells in an interphase condition. Eight hours after treatment with 6.26×10^{-4}M IAA began, the MI shows a decrease. This decrease was on the borderline of significance ($p=0.05-0.02$). On the basis of estimates of the duration of the G_2 phase (Howard and Pelc, 1953), the drop in MI in this period indicates an effect on G_2. In the following six hours mitosis is almost totally inhibited; the difference between the MI in treated and untreated roots is highly significant. The MI remains low for the following 12 hours and this indicates that there is a delayed inhibition in G_2 or that there was a direct inhibition in G_1 or S. We have already noted that an IAA-like auxin, NAA, inhibits DNA synthesis in lateral bud promeristems (Naylor, 1958), indicating that

auxin can act as an inhibitor of S. Since it appears that there is variation in the duration of G_1, S and G_2 (Clowes, 1961; Gelfant, 1962; Sisken, 1963), it is possible that even within eight hours of treatment part of the fall in the MI could be due to an inhibition acting before G_2.

Further evidence that IAA can prolong the duration of the mitotic cycle comes from results on the frequencies of polyploid cells. IAA treatments given with or following colchicine reduce the percentages of tetraploid cells subsequently seen in division. The reduction in the percentage number of tetraploid metaphases increases with increasing concentration of IAA. Unless this effect is a delayed one and operating only by preventing the entry of cells into mitosis, it appears that the reduction in the percentage of tetraploid metaphases is due to IAA affecting some stage which restitution nuclei must pass through before they can re-enter mitosis as tetraploid cells.

Since we have indicated that auxins can prevent DNA synthesis (Naylor, 1958) and that IAA can inhibit some process of interphase, it is interesting that the cells of the quiescent centre are, for the most part, in G_1. Clowes (1959 and 1963) has shown that the quiescent centre cells synthesize DNA before they enter division clearly indicating a delay in G_1. Though this appears to be the condition in most meristematic cells of the quiescent centre, some may be delayed in G_2;

Thoday's (1951) results suggest that some cells in V. faba roots have a G_2 of at least 72 hours.

It seems clear that there is still no direct evidence to support the view that IAA is a controlling factor in mitotic cycles or that the quiescent condition of some meristematic cells is induced by high concentrations of IAA. However, none of the evidence from several different approaches contradicts these suggestions and all the evidence supports them indirectly.

C. Colchicine and IAA.

Treatment with colchicine results in changes in the MI for a period of several days. In the first 24 hours the MI increases. Subsequently it falls to values below 1. Both the increase and the decrease in MI that follow treatment with colchicine can be modified by treatment with IAA.

Treatment with mixtures of colchicine and IAA prevent the initial increase in MI and with high concentrations of IAA (6.26×10^{-4} M) the increase is not only prevented but the MI is significantly lower than the controls. If roots are treated with IAA one day after treatment with colchicine, there is still a fall in MI but recovery to a nearly normal level of mitotic activity occurs sooner than in roots treated only with colchicine. These two results show that both effects induced by colchicine can be modified by IAA and recovery from the subsequent

depression of mitotic activity is enhanced by IAA. Both results suggest that the effects of colchicine on MI are due, at least in part, to changes in the concentration of some growth factor that can be replaced by IAA. The apparent antagonism between the effects of IAA and colchicine is also shown by the fact that colchicine prevents the severe inhibition of mitosis by high concentrations of IAA.

IAA also modifies the metaphase delay induced by colchicine. Though there is no evidence that IAA alone affects the duration of the stages of mitosis, it appears to reduce the metaphase delay that occurs in colchicine treated roots 24 hours after treatment (Table 5). The result of this effect is a drop in the number of metaphases. This reduction in the number of metaphases is reflected in changes in the prophase:metaphase ratio (Table 16).

The third system in which the effects of IAA and colchicine can be detected is root growth by elongation. Roots treated with colchicine do not grow after the first 24 hours (Levan, 1938; Witkus and Berger, 1950; Davidson, MacLeod and Taylor, 1965). Duhamet (1945) reported that treatment with a mixture containing 1.25×10^{-4}M colchicine and 1.14×10^{-12}M IAA did not inhibit root growth although 10^{-4}M colchicine alone blocked root growth completely. This result suggests that growth inhibition induced by colchicine is reversible with IAA. Similar results are reported for rhizome fractions using mixtures of colchicine and NAA (Martin, 1945). Roots treated with colchicine and subsequently with IAA or NAA also show greater growth than

roots treated only with colchicine (Levine and Lein, 1941; Levan and Lofty, 1949). In V. faba , treatment with 10^{-6} M IAA one day after colchicine treatment resulted in a stimulation of root growth (Davidson, MacLeod and Taylor, 1965). This stimulation was seen at four and six days after treatment and at both times was highly significant. Concentrations of IAA of this strength (10^{-6}M) are normally inhibitory to root growth and these results indicate that following colchicine treatment there was either a marked change in the sensitivity of the roots to IAA or a genuine stimulation of growth. As we have seen with MI, the addition of IAA appears to compensate for a deficiency of auxin in colchicine treated roots.

Similar results have been obtained in studies of crown gall tumours. It has been shown that X-ray induced inhibition of growth of the tumours can be reversed by IAA (Klein and Vogel, 1956). The tumour cells were also shown to require IAA for cell replication (Klein and Vogel, 1956) and since IAA synthesis is inhibited by X-rays (Gordon, 1956) it appears that radiation has reduced tumour growth by lowering the endogenous levels of auxin.

Conclusions

Roots treated with colchicine show three changes: their growth is inhibited, there is an increase and then a decrease in MI and there is a change in prophase:metaphase ratios due to metaphase delay. The

evidence presented here and from other systems shows that the effects induced by colchicine can be modified by treatment with IAA. The conclusion that has been drawn is that, following colchcine treatment, a change occurs in the level of growth factors in the root. Our results indicate that there is a progressive decrease in an IAA-like auxin in treated roots. It must be emphasized that the fall in auxin level is not immediate but takes place over a period of 48 or more hours. Thus, cell expansion continues for 24 - 36 hours after treatment with colchicine and MI does not reach minimal values for 48 - 60 hours. The fact that the MI increases before it decreases appears to indicate that the normal levels of IAA in roots act as a restraint on mitotic activity and, as IAA levels fall, that restraint is removed. When IAA levels fall below the stimulatory levels, IAA then becomes a limiting factor for cell division. As we have seen (Section 1 and this Discussion) both the rise and the fall in MI can be reversed by IAA.

The parallels between the effects induced by X-rays and colchcine indicate that the mechanism by which these effects are induced may be similar. In the case of X-rays it is known that auxin synthesis is inhibited; it seems probable that a similar event occurs following treatment with colchicine. Though IAA has been shown to restore growth in colchicine treated roots, it must not be thought that it is the only growth factor the synthesis of which is affected by colchicine. Other factors may also be affected.

SECTION TWO.

STUDIES OF CELL POLARITY.

Levan (1939) found that root elongation ceased altogether under the influence of colchicine, and that swellings or tumours formed at the root apex. Such tumours form in the zone of root elongation (Hawkes, 1942) apparently due to a change in the polarity of cell expansion. Root tumours are also induced in Allium by IAA (Levan, 1939) and as with colchicine they form in the zone of root elongation (Hawkes, 1942) largely due to the isodiametric expansion of the cortical cells. These swellings, the c-tumours, form in the absence (Levan, 1939) and in the presence of dividing cells (Davidson, 1961). IAA also caused a relative increase in the number of longitudinal and a decrease in the number of transverse division walls in the apical meristem of excised roots (Hughes and Street, 1960). Thus we see that a change in polarity in roots can be induced by IAA or it can occur, following treatment with colchicine, in the period in which it appears that there is a change in the level of endogenous growth factors in roots. Hawkes (1942) demonstrated that colchicine induces swellings to form in the zone of root elongation only when the root apex is present at the time of treatment.

Following colchicine treatment there is a change in the polarity of cell expansion and IAA has been shown to be implicated. Since the results in the previous section suggest that one result of

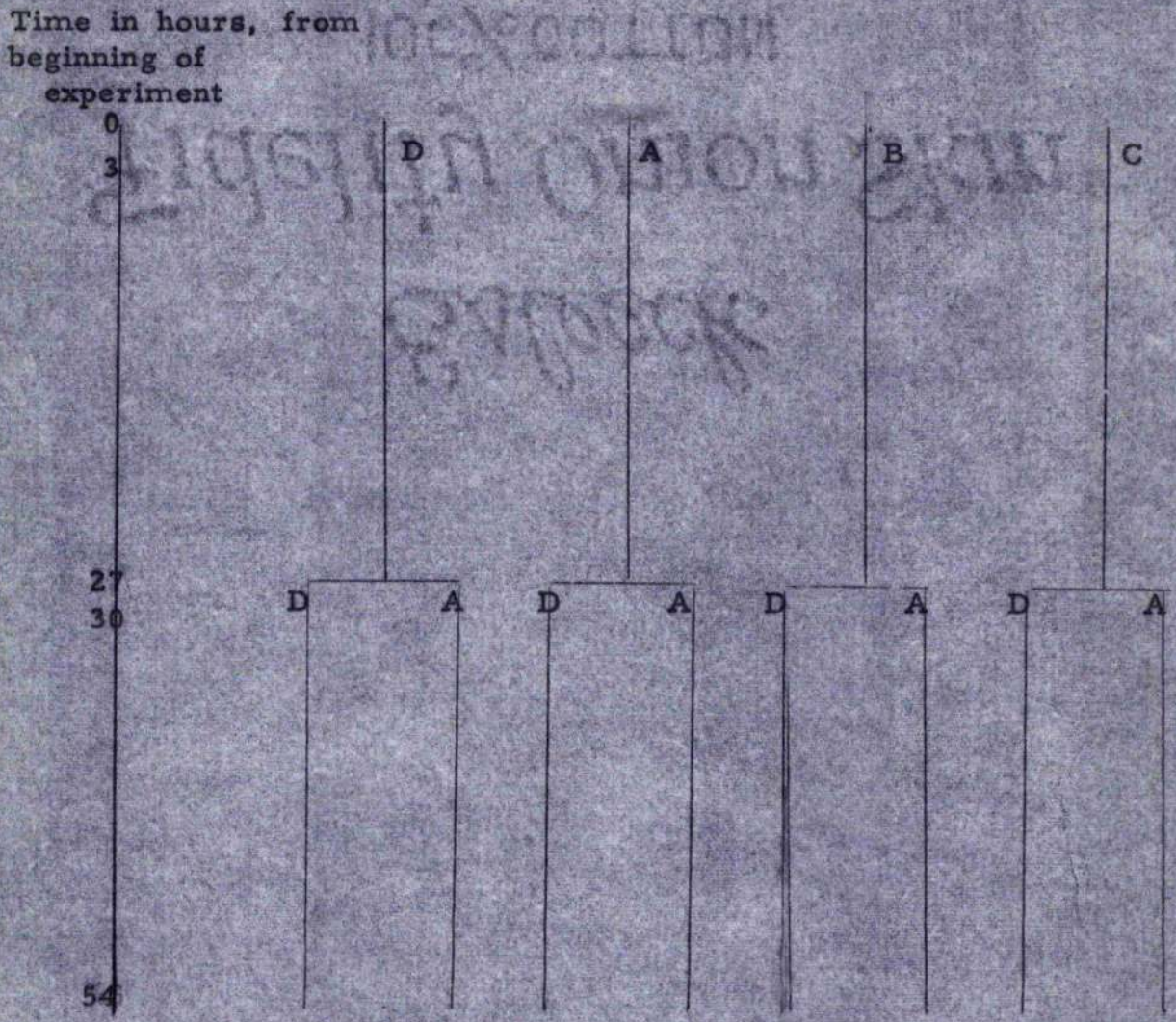
colchicine treatment is a change, in the level of growth factors, that can be reversed by IAA and since colchicine disrupts spindle formation, it was decided to determine the pattern of spindle orientation in normal roots and in roots treated with colchicine and IAA. Data was also obtained on the relative times at which normal cell division and root growth were re-established in treated roots. The results indicate that IAA did not affect the polarity of cell division in roots previously treated with colchicine. Other results to be reported here deal with the site of xylem differentiation and of the formation of lateral root primordia in the first 10,000 μ of treated roots.

Materials and Methods

Beans were grown in the manner described previously. Roots were treated for three hours with 4.7×10^{-6} M IAA and, or, 0.025% colchicine, according to the scheme laid out in Figure 13. One day intervals separated each treatment. Primary roots were fixed at the start of the experiment, 27 hours, 54 hours, 4 1/4 days, 7 1/4 days, 9 1/4 days and 11 1/4 days after the start of the experiment. The fixative was acetic-alcohol.

The roots were then embedded in wax and one 8 μ thick, 10 mm long median longitudinal section was made for each of five

Figure 13



A = a 4.7×10^{-6} M solution of IAA;

B = a 0.025% solution of colchicine;

C = a solution containing 4.7×10^{-6} M IAA and 0.025% colchicine;

D = controls, grown in the culture medium.

primary roots, giving a total of five sections for each treatment at each time of fixation. An E. Leitz 'Wetzlar' microtome was used to cut the sections. The sections were then stained with haemalaun (after Mayer), and permanent preparations were made.

The root apex in all measurements made is taken as the boundary between the root cap and the root apical meristem.

Results.

The numbers of cells in metaphase, anaphase and telophase and the orientation of these division figures with respect to the longitudinal axis of the root were recorded.

A. Control roots, grown in the culture medium.

In most, 79.1%, of the mitotic figures seen in metaphase, anaphase and telophase, the spindles are orientated in a plane parallel to the longitudinal axis of the root (Tables 22a). Spindle orientation could not be determined with certainty for a large number of metaphase figures. However, from the anaphase and telophase results, it is highly probable that most will later divide in a plane parallel to the longitudinal axis of the root.

A small number, 41, of metaphase, anaphase and telophase figures were orientated in a plane at right angles to the longitudinal axis of the root; i.e. radial divisions. These were in the apical

3270 μ. The plane of division of a small number of anaphase and telophase figures could not be determined with certainty. It is probable that most of these figures were dividing in a plane at right angles to both the longitudinal root axis and the plane at which the sections were cut; i.e. tangential divisions.

Most of the division figures recorded were in the apical 2070 μ of the root. The maximum number occurred between 0 and 1270 μ from the root apex. Cell division was distributed in all parts of the meristem. Outside the apical meristem, cell division gradually became predominantly confined to the pericycle and adjacent cells.

The cells of the apical 2470 μ of the root were arranged orderly in rows. Cell expansion began in the cells of the outer cortex between 470 μ and 870 μ from the root apex. This was followed by the expansion of the cells of the inner cortex. Vascular and stelar cell elongation began between 870 μ and 1270 μ from the root apex. At 2470 μ from the apex, the cortical cells had a rather irregular shape. This led to the development of intercellular spaces, thus making it difficult to observe any linear arrangement of these cells. Elongation of the stele had greatly increased by this point.

Xylem vessel elements had already differentiated between 1670 μ and 2070 μ from the apex of one root. However, the point at which such elements were first seen, varied greatly in different roots, and, in general, none were seen in the apical 5000 μ of the control roots. No lateral root primordia or polyploid cells in division were found in these roots.

B. Roots treated with IAA.

The results from studies of the plane of cell division in the treated roots are very similar to those obtained for the control roots (Tables 22b, e and f). The figures orientated to divide transversely to the longitudinal axis of the root appear to be, in general, 450 μ closer to the apex in the IAA treated roots than in control roots; the data, however, is based on a low number of such figures. Only one cell was seen in division in the root cap of the IAA treated roots and this occurred near the periphery of the root cap and the rest of the root.

The arrangement and development of the cells in the IAA treated roots was very similar to that of the control roots. The point at which xylem vessel elements were first seen lay between 2070 μ and 2470 μ from the apex. This point varied widely in different roots, as it did in the control roots.

Lateral root primordia were found in the IAA treated roots (Table 23). Dividing cells were found only in those primordia, which occurred at a distance of 5670 μ, or more, from the root apex. The distance between the primary root apex and the nearest lateral root has not been changed to any marked extent from the control value. No polyploid cells were found in the IAA treated roots, nor were any tumours obtained.

C. Roots treated with colchicine.

No cells in anaphase and telophase were seen, 24 hours after colchicine treatment (Table 22c). Anaphases and telophases were seen at 51 hours and in all subsequent fixations. Most of these anaphase and telophase figures are orientated parallel to the longitudinal root axis, as in the control roots.

There were many metaphase figures 24 hours after treatment and, as expected in c-metaphases, these figures were not orientated in any plane whatsoever. After a further 27 hours a few metaphase figures were orientated parallel to the longitudinal axis of the root and, after a further two days, the metaphase figures were orientated relatively normally, and remained so for the duration of the experiment.

The orientation of a few anaphase and telophase figures could not be determined, as with the control roots. These figures are

probably orientated tangentially. No figures showed a radial orientation for the initial 51 hours after treatment. Subsequently, however, such figures were observed in this plane. Division figures showing a radial orientation are localized predominantly in the apical 470 μ of the root, and in the lateral root primordia and lateral roots, which have developed since treatment. Two division figures orientated in this plane were recorded outside these tissues. One of these figures was between 1670 μ and 2070 μ from the root apex. However, such figures were found in the control roots in this part of the root. The other was found between 4870 μ and 5270 μ from the root apex. This figure may represent one of the initial division figures in lateral root primordium formation (Tables 22a and c).

Most of the division figures were in the apical 2070 μ of the root, 24 hours after treatment, and in the apical 870 μ at all subsequent times. Thus we see that the region in which cell division occurs is smaller following treatment with colchicine; it seems significant that this contraction in the size of the active mersitem should occur in a period in which we have already shown that there is a marked reduction in MI (Section 1, part 4). A few cells were seen dividing in the root cap at the periphery of the root cap with the rest of the root.

For the first 51 hours after colchicine treatment, the cells of the root apex were linearly arranged, as in the control roots. After a further 48 hours these cells were not arranged in such an orderly fashion (Figure 16). After a further five days, the normal linear arrangement of the root apical cells could no longer be seen, and the cells had become elongated and spindle-shaped. Two days later some of the roots had begun to regenerate. The cells in the apical 470 μ of these roots were now linearly arranged. Such a linear arrangement of the cells in the root apex could not be seen in the roots which had still not begun to regenerate.

Colchicine induces the formation of polyploid cells. Such polyploid cells can be seen in the apical 470 μ of the roots. They contribute to the disorder in the apex found after treatment, because they are larger than diploid cells and they upset the normal arrangement of the cells in the root apex (Figure 16).

When order is reestablished in the root apex, rows of large and small cells can be seen, i.e. rows of polyploid cells and diploid cells can be seen (Figure 17). This is evidence that both diploid and polyploid cells are present in the new meristem, which forms in the root apex after colchicine treatment and this meristem consists both of cells, which were affected by the colchicine, and cells which were not affected.

Cortical cell expansion begins between 870 μ and 1270 μ from the root apex and is at first quite normal. However, these cells expand greatly in the sub-apical region of the root, i. e. principally between 3270 μ to 5670 μ, and produce the root tumours. Elongation in the stele also begins between 870 μ and 1270 μ from the root apex, and continues even in the root tumour. Differentiated xylem vessel elements were first seen between 2470 μ and 3270 μ from the apex. Eleven days and three hours after treatment, however, such elements were found in the apical 1270 μ of the root, between 870 μ and 1270 μ from the apex. The level at which xylem vessel element differentiation occurs in the colchicine treated roots is closer to the apex than in the controls.

Lateral root primordia were first seen 4 days and three hours after treatment (Figure 18). Thereafter such primordia were found in all of the roots examined (Table 23). These primordia were mainly in the c-tumour. Other primordia were found, however, outside the c-tumour. For the initial seven days and three hours after treatment, most of these primordia contained dividing cells. Thereafter, no dividing cells were seen in many of these primordia. All these primordia contained polyploid cells. This is to be expected as these primordia form from pericycle cells, some of which were probably in division

at the time of colchicine treatment. Small lateral roots were seen above the c-tumour eleven days and three hours after treatment. These lateral roots were morphologically normal. Polyploid cells were not seen in the apical meristem of these lateral roots, but such cells did occur more basally along these roots. This means that polyploid cells were included in the apical meristem at the time it first formed and were later lost. It cannot be determined from the available evidence which of these hypotheses is correct.

D. Roots treated with colchicine and subsequently with IAA.

Most, 75.6%, of the anaphase and telophase figures and some, 21.5%, of the metaphase figures are orientated parallel to the longitudinal axis of the root (Tables 22d). A number of metaphase figures, 87, were unoriented for the initial 72 hours after treatment with IAA. The number of such unorientated cells found was far less than that found after treatment with colchicine alone (Table 22c). This was especially true 54 hours after the experiment began, e.g. in the roots treated only with colchicine 131 metaphases were unorientated, compared with 47 in the roots treated with colchicine and subsequently with IAA. These results reflect the IAA induced reversal of the mitotic stimulation found after colchicine treatment (Tables 13i and n; Figure 11; Section 1 Part D). Many of the

metaphase-telophase figures orientated radially to the root longitudinal axis were in the apical 1270 μ of the root (65.5%). Outside this region, cell division occurred in the pericycle; these divisions were involved in primordia formation. No dividing cells were found in the root cap.

The root cells were linearly arranged, as in the control roots, for the first three days after treatment with IAA. After a further three days, a linear arrangement of these cells could not be clearly seen because many of the cells had become elongated and spindle-shaped. Once the roots began to regenerate the normal pattern of cells was re-established in the apex.

Elongation in the stele began between 870 μ and 1270 μ from the root apex and cell expansion in the cortex began between 870 μ and 1270 μ from the root apex. Tumours were found on all of these roots but they were generally smaller than those obtained after treatment with colchicine alone. These tumours formed as a result of the isodiametric expansion of the cells in the cortex. Eight days after IAA treatment, vessel elements of xylem were seem between 470 μ and 870 μ from the root apex in the roots which still had disorganised apices (Figures 19 and 20). In roots with normal apices, however, they were seen between 4070 μ and 4470 μ from the root apex (Table 23).

Lateral root primordia were found in these roots (Table 23). These primordia developed from the pericycle cells and they contained polyploid cells. Some of these primordia had no dividing cells. A few lateral roots also developed after treatment and these also contained polyploid cells (Table 23).

E. Roots treated at the same time with colchicine and IAA.

Unlike the roots treated with colchicine alone, anaphase and telophase figures are present 24 hours after treatment in roots treated with a solution containing colchicine and IAA (Tables 22g and h). Such figures are predominantly (62.2%) orientated with respect to the longitudinal axis of the root, 24 hours after treatment with a mixture containing colchicine and IAA. Three days and three hours later, spindle orientation is more nearly normal, especially in the roots treated with IAA subsequent to treatment with IAA and colchicine. At this time and thereafter many (41.4%) of the metaphase figures were orientated parallel to the longitudinal axis of the root, and 16.4% were orientated radially to the longitudinal axis of the root, In some cells the chromosomes were in the metaphase condition but the cells were not; spindle orientation could not be determined. Many (43.6%) of the figures orientated to divide radially to the longitudinal root axis are in the apical 870 μ of the

root. At times of 174 or more hours from the beginning of the experiment, 70% of the figures orientated radially to the longitudinal axis of the root outside the apical 870 μ are associated with primordia formation (Tables 22g and h and 23).

Most (89.8%) of the dividing cells were in the apical 2070 μ of the root 27 hours after the experiment began (Table 22g). Gradually a greater percentage of dividing cells were distributed more apically in the root, e.g. 270 hours after the beginning of the experiment 85.7% of the dividing cells were in the apical 270 μ of the root (Table 22h). It appears, therefore, that the size of the meristem is influenced by IAA; when little IAA is present, the root apical meristem is larger than when IAA is more plentiful. Only one cell was seen in division in the root cap. This cell was on the periphery of the root cap and the rest of the root.

For the initial 54 hours after the experiment began, the root cells were linearly arranged as in the control roots. Within a further 48 hours, however, it was difficult to see a linear arrangement of the cells in the apical 870 μ of the root, as the cells had become spindle-shaped. After eleven days and six hours, some of the root apices were structurally normal, but others were still disorganised. Thus, some roots recover faster than others and this is seen in the arrangement of the cells in the root apex. In

one disorganised apex, i. e. an apex in which the arrangement of the cells was disorganised, there was a primordium in the apical 470 μ of the root. Cell division in the apex of this root was confined to this primordium.

Elongation in the stele and cortical cell expansion began between 470 μ and 870 μ from the root apex. Expansion of the cortical cells continues in the more basal parts of the root and the cells become irregular in shape. This leads to the development of intercellular spaces and to the disruption of the linear arrangement of the cells. At about 1270 μ and more basally along the root from the apex the cortical cells begin to expand markedly and produce the c-tumour. Though the expansion of the cortical cells is abnormal, i. e. isodiametric rather than polarised, in the tumour region no effect on the stelar cells was found. Even in the tumour the stelar cells continue to elongate rather than to expand isodiametrically. This may be due to cells in the outer part of the root preventing the isodiametric expansion of cells in the inner part of the root by physical pressure. An alternative explanation is that the stelar and cortical cells show different levels of sensitivity to colchicine; one expands isodiametrically, the other does not.

Differentiated xylem vessel elements were found between 870 μ and 1270 μ, seven days and six hours after the experiment began, in the roots treated with colchicine and IAA at the start of the experiment. The point at which differentiation of these elements first took place, however, varied with time, in both these roots and the roots which were also treated with IAA, 27 hours after the experiment began, e.g. in the former roots, differentiated xylem vessel elements were first seen between 3270 μ and 3670 μ from the root apex, 54 hours after the beginning of the experiment, and between 870 μ and 1270 μ from the apex, 120 hours later (Table 23). Differentiation, therefore, appears to depend on cell age, i.e. as cells get older, they differentiate.

Lateral root primordia first developed 54 hours after the experiment began. The primordia were found in most of the roots examined in later fixations (Table 23). In general, dividing cells were seen in these primordia only in the first four days and six hours after the experiment began; few, or no, dividing cells were seen in the primordia after this time. Lateral roots were seen to grow from roots treated with colchicine and IAA at the start of the experiment, nine days and six hours after the experiment began (Table 23). Polyploid cells were present in all of these primordia and lateral roots.

Discussion.

1. Polarity.

The apical meristem of the root consists of a cup-shaped group of cells, known as the apical initials. These cells surround a group of several hundred cells, known as the quiescent centre (Clowes, 1961). The apical initial cells give rise to all the root tissues. Most of the root growth takes place in the apex, partly in the meristem and partly in the region immediately promixal to the meristem. The cells of the apical meristem divide and grow at rates which maintain their average volume more or less constant.

The pattern of cell division of these apical initial cells is of great importance, since it will determine the shape of the root and the positions of the different tissues with respect to each other. The axis of each cell division coincides with that along which the two daughter cells will expand in tissues where both cell expansion and division are only in one direction. In gourds, it has been shown that there is a definite relation between the orientation of the mitotic figures and the direction of growth in these organs (Sinott, 1944). Thus, the plane of cell division is related to the direction of growth. Cell wall formation between daughter cells is at right angles to the axis of the telophase figure. This

relationship is less exact in earlier phases of mitosis, because the figure probably rotates and does not settle down until telophase (Sinott, 1944).

In the median longitudinal sections of roots, lines of cells are seen radiating from a region at the apex of the root. Where a small line of cells was continuous with a double line, the junction was produced by a cell dividing transversely, followed by the division of one of the daughter cells by a longitudinal wall, i.e. there is a division in a plane parallel to the longitudinal axis of the root, followed by a division in a plane at right angles to this axis in one of the daughter cells.

Increase in diameter of the root near the apex is due only to an increase in the number of rows of cells by the occasional cell division at right angles to the root longitudinal axis: the cells remain fairly constant in size. Further away from the apex, towards the more basal regions of the root, increase in root diameter is due both to cell expansion and to cell divisions that lead to an increase in the number of cells making up the diameter of the root. In the most basal regions of the roots examined in this study all increase in the root diameter was due to cell expansion. The only cells outside the apical 600 μ of the root which underwent no cell expansion were those of the pericycle. Thus the polarity of root expansion depends initially on the plane of cell division,

later, on both the plane of cell division and that of cell expansion and, finally on the plane of cell expansion alone.

The expansion of cells that produce visible root growth occurs outside regions that are centres of active mitosis. If we confine our attention to apical meristems we see that a region of active division and no major expansion is succeeded by a region with little mitotic activity and marked cell expansion. This could be explained by a gradient of some growth factor along the root. However, such a mechanism ignores the presence of expanding cells in the epidermis of the meristem and the meristematic cells of the pericycle. This suggests that cell expansion and division are not controlled by a single growth factor but that the different phases of growth are controlled by extrinsic and intrinsic factors. In cell expansion and in cell division, one problem common to both systems is to define the mechanism that determines polarity - in mitotic cells the polarity of spindle orientation and in growing cells the axis of elongation.

It is difficult, for example, to envisage completely extrinsic control of the polarity of spindle orientation and cell division. For division figures in the meristem are at right angles to one another, i.e. not all mitotic figures are orientated along one axis. Thus, it appears that the polarity of cell division is

controlled, at least to some extent, by some internal factor of the cell. Each plant species has a distinct root pattern. This suggests that the plane in which a cell divides must also bear some relation to the plane of division of the other root cells. This may be a mechanical or a chemical relationship, or some combination of the two.

Bloch (1943) has discussed the effects of external stimuli on cell polarity. Though single treatments are able to affect the polarity both of division and cell expansion it remains to be seen whether they act through an effect on a common mechanism. IAA is an example of a growth factor that disturbs the polarity of cell expansion (Levan, 1939; Carlton, 1943) and cell division (Burström, 1942; Mohr, 1956). Burström (1942) noted that in wheat roots, IAA changed the plane of division of some cells from a tangential to a radial plane. In his experiments and those of Mohr (1956) the result of disturbing the polarity of cell division was the production of disorganised tissue.

In roots of V. faba treated with 4.7×10^{-6} M IAA, no effect on the polarity of cell division or the pattern of organisation of cells in the root apex was seen (Tables 22b, e and f). Following colchicine treatment cell division is suppressed, apparently due to the absence of anaphase and telophase. When spindles reappeared,

their orientation appeared to be normal (Tables 22c) and there was no evidence of a disruption of the polarity of cell division, even though the roots showed no growth. Treatment with IAA following, or with, colchicine produced no change in spindle orientation. Thus, there is no evidence from these results that IAA affects spindle orientation in V. faba roots.

After colchicine treatment, root elongation stops and tumours form in the zone of root elongation (Levan, 1938; Hawkes, 1942). These tumours are produced as a result of isodiametric expansion of cortical cells (Hawkes, 1942). Roots, the growth of which has been inhibited by colchicine, eventually regenerate (Levan, 1938; Witkus and Berger, 1950; Davidson, 1961, 1965b; Davidson, MacLeod and Taylor, 1965). In the present experiments root regeneration did not begin until about eleven days after treatment with colchicine. Therefore, there can be normal polarity of spindle orientation even though the roots are not growing and it may be that elongation and spindle polarity are not controlled by the same mechanism.

It has been found that the change in the polarity of cell expansion induced by colchicine can be modified by IAA (O'Riordan, 1965). Thus, it appears from the studies of the effects of IAA

on colchicine treated roots that, though IAA can modify the effects of colchicine and can stimulate the recovery of inhibited roots, there is no evidence that IAA is affecting the polarity of the spindle.

B. Primordia formation.

Goldacre (1959) found that a 10^{-5} M solution of IAA promoted the formation of lateral root primordia from the pericycle cells of isolated flax roots. Such primordia did not elongate and form lateral roots unless the roots were transferred to an IAA free medium. These results are in agreement with the results reported here. Lateral root primordia formation was stimulated by 4.7×10^{-6} M IAA, but there was no affect on the spatial distance between the root apex and the nearest erupted lateral root, compared with the controls. No polyploid cells were found in these primordia, or in division in the rest of the root. This indicates that IAA, in the concentration used, has not induced polyploidy nor has it induced any polyploid cells present in the root to come into division.

Lateral root primordia formation is stimulated following colchicine treatment. In some cases, these primordia developed into lateral roots. These lateral roots grow out from the apical

10 mm of the root. Thus, the lateral root primordia, which develop as a result of colchicine treatment, develop normally, unlike those which form after IAA treatment.

Primordia also form after treatments with both IAA and colchicine. In the roots treated with colchicine and IAA at the same time or at different times, these primordia later grew out as lateral roots. In the roots treated with IAA and colchicine at the same time and 24 hours later, with IAA, no lateral roots grew out. Lateral root primordia formed closer to the primary root apex following IAA treatments, whether colchicine was present or not, than in roots treated only with colchicine. Some of the lateral roots produced by colchicine treated roots grow from the c-tumour. These laterals and those originating outside the c-tumour contained polyploid cells. The primordia and lateral roots which formed after colchicine treatment contained polyploid cells irrespective of the presence of IAA. Therefore, these tissues must have developed, at least partly, from cells which were affected by the colchicine treatment. No estimates were made of the frequencies of polyploid cells in these roots, though it is known that polyploid cells can occur in high frequency in such roots (Davidson, 1961; 1965a and b).

IAA stimulates primordia formation (Table 23) both in roots that were and were not also treated with colchicine. It is clear, however, that though IAA stimulates primordia formation, it seems to be inhibitory to the development of these primordia into lateral roots. Even in the roots treated with colchicine and IAA at the same time and, 24 hours later, with IAA, no lateral roots develop from the primordia which have formed as a result of the treatment. It appears that primordia become sensitive to IAA after a certain stage in their development and their growth is then inhibited. Similar results have been reported for growth of lateral roots of Vicia treated with colchicine and then IAA (Davidson, MacLeod and Taylor, 1965). Thus exogenous IAA is a necessary factor for lateral root primordial initiation but not for primordial growth.

It can be seen (Table 23) that differentiated xylem vessels occur closer to the apex in colchicine treated roots than in the controls. This differentiation is not prevented by IAA and even treatments with IAA alone lead to some xylem differentiation closer to the apex than it occurs in the controls (Table 23). Bhaduri (1939) found that colchicine treatment seems to accelerate the differentiation of vascular tissue, because the region of differentiation extends closer to the apex of colchicine treated roots. However,

it seems more probable that xylem differentiation occurs closer to the root apex in the colchicine treated roots than in the controls because colchicine treated roots have stopped growing and differentiation is a function of the age of the tissue. The pattern of vascular differentiation is determined by the apex of the root and not by any existing pattern in the more mature parts of the root (Bünning, 1952; Torrey, 1955). Furthermore, the differentiation of xylem from undifferentiated tissue is under the control of IAA (Torrey, 1957; Fosket and Roberts, 1964).

Lateral root primordium formation appears to be associated with vessel differentiation. This is a consequence of the fact that primordia do not form closer to the primary root apex than differentiated xylem vessel elements, or, at least xylem vessel elements are always present in the region of the root where primordia formation is taking place. Lateral roots are initiated opposite xylem vessel elements in normal V. faba roots (Goldacre, 1959). This suggests that it is some factor (s) transported in the xylem vessels which is responsible for the development of these primordia.

In the control roots cell division is not localised at the apex, but occurs in all parts of the meristem. Outside the meristem, cell division is confined to the pericycle and adjacent cells. These

dividing cells do not expand. After treatment with colchicine and IAA, or colchicine alone the cells of the apical meristem become elongated. Therefore, these treatments must have upset the level of some factor which normallyprevents the expansion of dividing cells. de Ropp (1956) has shown that kinetin suppresses the IAA induced elongation of apical sunflower fragments. Kinetin is a necessary factor for cell division (Miller, Skoog, von Saltza and Strong, 1955). If it is produced by dividing cells, then, after treatment with colchicine it may be that levels of a kinin are reduced for there is a reduction in the MI after colchicine treatment. In the absence of kinetin, cell expansion would occur. Cell expansion does not occur for several days after treatment. Therefore, any change in the level of a kinin in the root does not occur immediately.

D. Regeneration.

Order has been re-established in roots after treatment with colchicine, or colchicine and IAA, once the linear arrangement of the cells in the root apex is seen. Rows of polyploid and diploid cells can be seen (Figure 17). Therefore, both polyploid and diploid cells are present in the new meristem which forms after treatment and both are producing lineages of cells. This shows

that the new meristem consists of cells which were affected by the treatment, as well as cells which were not. Thus, the new meristem has formed from cells of the old meristem and, perhaps, from cells, of the quiescent centre, which were not dividing at the time of treatment. Quiescent centre cells divide rarely (Clowes, 1956) and therefore will not be affected by the colchicine treatment, i.e. these cells will not become polyploid. The new meristem may form in the same position as the meristem which was present at the time of treatment, or it may form in a slightly different position.

SECTION THREE

GENERAL DISCUSSION

General Discussion

Following treatment with 0.025% colchicine, changes occur in the MI of root meristems; within about 24 hours there is a significant increase in MI and this is followed by a decrease until, three days after treatment, the MI is less than 1. Other changes also occur. A delay is induced at metaphase and results in a shift in the prophase : metaphase ratio. This metaphase delay persists even after normal anaphases reappear, suggesting that it is not due solely to the prevention of normal anaphase movement of chromatids. Restitution also occurs and leads to the formation of polyploid cells. Polyploid cells seen in division 24 hours after treatment indicate that the delay induced in metaphase is not so prolonged that it extends the intermitotic time, i.e. the duration of the mitotic cycle in these polyploid cells has not been extended (cf. Howard and Pelc, 1953). Since the appearance of polyploid cells in mitosis coincides with the increased MI, it seems that metaphase delay is not solely responsible for this increase. Furthermore, if metaphase delay was responsible for the increase in MI, the increase should be maintained as long as there is evidence of metaphase delay; this was not found to occur. The MI is low, three days after treatment, even though the prophase : metaphase ratio indicates metaphase delay.

The increase in MI induced by colchicine is reversed by IAA. One effect of IAA is to reduce metaphase delay and, with slightly higher concentrations, there is a reduction in the number of cells in prophase. The inference that can be drawn from these results is that IAA is inhibitory during interphase and this inference is supported by the ability of IAA to delay the entry of polyploid cells, which act as a marked population of cells, into mitosis.

The hypothesis on which the experiments developed in this thesis were based is this: that following colchicine treatment there is a decrease in the level of growth factors in the root, that this decrease is responsible for changes in MI, growth and patterns of differentiation and that the change occurs over a period of several days.

On the basis of this hypothesis it would be expected that the responses of roots to colchicine would be modified by exogenous sources of growth factors. This has been found to be so. IAA affects the changes in MI, both inhibiting the initial increase and facilitating recovery from the subsequent decrease; it alters the pattern of lateral root initiation in colchicine treated roots and it also changes the size of the functional meristem. It had previously been shown that IAA stimulates the growth of roots previously inhibited by colchicine (Davidson, MacLeod and Taylor, 1965).

Root growth by elongation ceases after colchicine treatment even though dividing cells continue to occur in the treated roots (Davidson, 1961). This suggests that mitotic inhibition is not responsible for cessation of root growth and that mitotic activity and growth are not affected to the same extent by changes in levels of growth factors. Though mitotic activity is maintained in some cells it is not maintained in all the meristematic cells present at the time of colchicine treatment and the functional meristem appears to shrink (Section 2). This contraction of the meristem can be prevented in part by IAA. There is evidence that cell polarity can be changed by IAA (Burström, 1942; Mohr, 1956) but it appears that the changes induced by colchicine are not reversed by IAA.

The changes that occur in meristems following treatment with colchicine are very similar to those that follow irradiation; mitosis is inhibited, the region of active meristematic activity contracts (Gray and Scholes, 1951) and root growth is inhibited (Gray and Scholes, 1951). The re-establishment of meristematic activity is correlated with the onset of active mitotic activity in cells of the quiescent centre (Clowes, 1959, 1961, 1963). X-rays are known to inhibit IAA synthesis (Gordon, 1956). This fact, together with the auxin gradient present in roots (Pilet, 1951) and

and the high sensitivity of the apical meristem to X-rays, suggests that some synthesis of auxin-like growth factors occurs at the apex of the root. The parallels between the effects induced by colchicine and X-rays appear to lend support to the view that colchicine disrupts the synthesis of growth factors. Many observations have been made of the effects of treatments with colchicine and IAA or some other auxin (see Discussion, Section 1); no result that has been reported so far contradicts the hypothesis that colchicine leads to changes in the level of growth factors in roots. No suggestion can be made at present concerning the mechanism by which colchicine affects auxin synthesis. Further experimental analysis of roots treated with colchicine and auxins should provide crucial evidence for the bases of normal and abnormal growth.

SECTION FOUR

SUMMARY AND CONCLUSIONS

Summary and Conclusions

The effects of colchicine and IAA on roots of Vicia faba L. have been examined; changes were found in mitotic index, polarity and differentiation. The following conclusions were reached:

1) IAA suppresses or delays some process(es) in the G_2, S and probably also the G_1 stages of interphase. IAA has no effect on mitosis, but because of its effects on interphase, causes a decrease in the MI. IAA does not appear to be a factor involved in spindle formation, and the time at which spindles reappear after colchicine treatment is not influenced by IAA.

2) Colchicine blocks the anaphase separation of the chromatids. This is not due to a change in pH and it does not involve IAA. Because colchicine blocks the anaphase separation of the chromatids, restitution takes place and polyploid cells are seen in division one mitotic cycle later. The duration of metaphase is apparently increased after colchicine treatment.

3) A result of colchicine induced metaphase delay is that the relative duration of metaphase is lengthened and a change occurs in the prophase : metaphase ratio in favour of metaphases, compared with the control values. This change in the prophase : metaphase ratio is reversed by IAA, many hours after treatment, to produce prophase : metaphase ratios similar to those of the control roots. IAA has this effect for at least two reasons; the relative duration of metaphase is shortened from the value found after treatment with colchicine, to a value similar to that of the controls and, the percentage of polyploid metaphases found after colchicine treatment is lowered by treatments with both IAA and colchicine.

4) Following colchicine treatment, changes occur in the MI; there is a significant increase 24 hours after treatment but after a further three days the MI is less than 1. These effects of colchicine on MI can be changed by IAA; 4.2 x

10^{-4} M IAA in a mixture containing 0.025% colchicine prevents the MI from changing significantly from the control values, 24 hours after treatment and 4.7×10^{-4} M IAA, given 24 hours after colchicine, facilitates the recovery of the MI to values, which are not significantly different from those of the controls, several days after treatment. Treatment with 4.2 or 4.7×10^{-4} M IAA results in an inhibition of mitosis 24 hours later. This inhibition is prevented if 0.025% colchicine is also present at the time of treatment. These results indicate that the changes in MI which are found following treatment with colchicine are associated with a change in the level of growth factors that normally control mitosis in the root and that this change takes place over a number of days.

5) Polarity of root growth is initially determined by the plane of cell division. In more basal regions of the root, it is determined by both the plane of cell division and that of cell expansion. Finally, it is determined by the plane of cell expansion alone.

6) IAA induces lateral root primordia formation, but these fail to develop normally, however, unlike the primordia that appear in the apical 10 mm of roots treated with colchicine and which grow out as lateral roots.

7) The polarity of cell division is re-established in colchicine treated roots as soon as normal mitosis reappears. Thus, one aspect of root polarity returns to normal several days before cell elongation recommences. IAA does not affect the polarity of cell elongation or division in the concentration used.

8) The cessation of root growth after colchicine treatment is not due to the cessation of cell division, but to the altered polarity of cell division.

9) Differentiation of xylem vessel elements occurs closer to the apex in colchicine treated roots than in the controls. IAA does not prevent this differentiation. In roots treated only with IAA xylem elements are seen closer to the apex compared with the control roots.

10) All of the primordia that develop after colchicine or colchicine and IAA treatments do so, at least partly, from cells affected by the colchicine. This is also true of the new meristem which forms, after these treatments, in the root apex. The new apical meristem forms partly from the cells of the meristem which was present at the time of treatment and partly from the cells of the quiescent centre.

11) IAA does not induce polyploidy, nor does it induce polyploid cells present at the time of treatment to divide.

12) The response of roots to IAA treatment, with respect to MI, depends on the age of the root at the time of treatment.

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FLUORESCENT

APPENDIX ONE.

TABLES.

Bolloco

Fidelity Onion Skin

100% COTTON

FLUORESCENT

Table I

Treatment	pH of solution	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	Abnormal Anaphase and Telophase	MI
water (control)	6.49	5484	340 (65.9)	82 (15.9)	26 (5.0)	68 (13.2)	-	8.6
0.025% colchicine	7.85	5529	332 (70.5)	104 (22.1)	-	-	35 (7.4)	5.9
0.021% colchicine and 6×10^{-3} M HCl	3.82	5399	469 (78.0)	72 (11.9)	-	-	60 (10.0)	10.0
0.021% colchicine and 1.04×10^{-4} M IAA	4.22	5481	402 (77.5)	91 (17.6)	-	-	26 (5.1)	8.6
6.26×10^{-4} M IAA	3.90	5368	476 (75.3)	68 (10.8)	36 (5.7)	-	52 (8.2)	10.5
10^{-4} M HCl	4.08	5665	243 (72.5)	54 (16.1)	21 (6.3)	17 (5.1)	-	5.6

Legend Table 1. Numbers of cells in interphase and in mitotis in cells of Vicia faba roots treated with various combinations of colchicine, IAA and HCl for 3 hours. Each determination is based on 6,000 cells; 600 cells were scored from each of 10 roots. The figures in brackets are the percentages of cells in different stages of mitosis. The concentrations of colchicine, IAA and HCl given are the concentrations in the mixtures.

Table 2

Colchicine	IAA*	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
−	-	5724	178	42	8	48	4.6
+	-	5527	148	325	-	-	7.9
+	0.329	5531	223	246	-	-	7.8
+	0.625	5594	129	277	-	-	6.8
+	1.043	5450	128	422	-	-	9.2
+	2.083	5466	156	377	-	1	8.9
+	3.13	5493	197	310	-	-	8.5
+	4.2	5566	157	277	-	-	7.2
+	4.7	5564	163	273	-	-	7.3
+	5.2	5645	116	239	-	-	5.9
+	5.6	5642	144	214	-	-	6.0
+	6.26	5618	145	237	-	-	6.4
−	0.329	5685	213	50	7	45	5.3
−	0.625	5799	137	30	9	25	3.4
−	1.043	5695	200	54	5	46	5.1
−	2.083	5789	137	46	8	20	3.5
−	3.13	5770	124	82	8	16	3.8
−	4.2	5800	126	52	5	17	3.3
−	4.7	5767	141	50	2	40	3.9
−	5.2	5795	121	40	18	26	3.4
−	5.6	5769	145	44	7	35	3.9
−	6.26	5698	208	40	6	48	5.0

* x 10^{-4} M

Legend Table 2. Numbers of cells in interphase and in mitosis in cells of V. faba roots treated with colchicine and, or, IAA for 3 hours. In all treatments with colchicine the final solution contained 0.025%. The values for IAA are from 0.329 to 6.26×10^{-4} M. Each determination is based on 6,000 cells; 600 cells were scored from each of 10 roots.

Table 3

Colchicine	IAA*	Prophase	Metaphase	Anaphase	Telophase
-	-	64.5	15.2	2.9	17.4
+	-	31.2	68.9	-	-
+	0.329	47.5	52.5	-	-
+	0.625	31.8	68.2	-	-
+	1.043	23.3	76.7	-	-
+	2.083	29.2	70.6	-	0.2
+	3.13	38.9	61.1	-	-
+	4.2	36.2	63.8	-	-
+	4.7	37.4	62.6	-	-
+	5.2	32.7	67.3	-	-
+	5.6	40.2	59.8	-	-
+	6.26	38.0	62.0	-	-
-	0.329	67.7	15.9	2.1	14.4
-	0.625	68.2	14.9	4.5	12.4
-	1.043	65.6	17.7	1.6	15.1
-	2.083	64.9	21.8	3.8	9.5
-	3.13	54.0	35.7	3.4	6.8
-	4.2	63.0	26.0	2.5	8.5
-	4.7	60.5	21.5	0.9	17.2
-	5.2	59.0	19.5	8.8	12.7
-	5.6	62.8	19.1	3.0	15.2
-	6.26	68.9	13.3	1.9	15.9

* $\times 10^{-4}$ M

Legend Table 3. Percentages of cells in different stages of mitosis in roots of V. faba treated with colchicine and, or IAA. Based on data in Table 2.

Table 4

a) 3 hours after treatment began:		
control and 0.025% colchicine	$p = 0.10 - 0.20$	Not significant
0.025% colchicine and the mixture containing 0.025% colchicine and 0.3×10^{-4} M IAA	$p > 0.90$	Not significant
control and the mixture containing 0.025% colchicine and 0.3×10^{-4} M IAA	$p = 0.10 - 0.20$	Not significant
b) 24 hours after treatment ended:		
control and 0.025% colchicine	$p < 0.001$	Significant
0.025% colchicine and the mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA	$p < 0.001$	Significant
control and the mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA	$p > 0.50$	Not significant
control and 4.2×10^{-4} M IAA	$p < 0.001$	Significant

Legend Table 4. Probability values for significance of differences between pairs of mitotic indices after various treatments with colchicine and, or IAA. Probability values were calculated by the t test. The original data were given in tables 2 and 5.

Table 5

Colchicine	IAA*	Inter-phase	pro-phase	meta-phase	ana-phase	telo-phase	MI
–	-	5583	231	84	36	66	6.95
+	-	4969	212	818	-	1	17.2
+	0.329	5059	284	654	-	3	15.7
+	1.043	5194	125	677	-	4	13.4
+	2.083	5297	143	559	-	1	11.7
+	3.13	5719	138	143	-	-	4.7
+	4.2	5639	198	163	-	-	6.0
+	4.7	5805	63	131	-	1	3.3
+	5.2	5937	33	30	-	-	1.1
+	5.6	5940	47	13	-	-	1.0
+	6.26	5970	20	10	-	-	0.49
–	0.329	5592	262	80	36	40	6.95
–	0.626	5687	179	70	18	46	5.2
–	1.043	5683	193	59	18	47	5.3
–	2.083	5713	182	60	13	32	4.8
–	3.13	5958	30	10	1	1	0.7
–	4.2	5944	31	21	1	3	0.9
–	4.7	5880	74	18	6	22	2.0
–	5.2	5860	75	41	3	21	2.3
–	5.6	5897	52	24	6	21	1.7
–	6.26	5962	3	31	-	4	0.63

* x 10^{-4} M

Legend Table 5. Numbers of cells in interphase and in mitosis in cells of V. faba roots treated with colchicine and, or, IAA for 3 hours and fixed after 24 hours recovery. 600 cells were scored from each of 10 roots.

Table 6

Colchicine	IAA*	Prophase	Metaphase	Anaphase	Telophase
–	–	55.4	20.1	8.6	15.8
+	–	20.6	79.3	-	0.1
+	0.329	30.2	69.5	-	0.3
+	1.043	15.5	84.0	-	0.5
+	2.083	20.3	79.5	-	0.1
+	3.13	48.0	52.0	-	-
+	4.2	54.8	45.2	-	-
+	4.7	32.3	67.2	-	0.5
+	5.2	52.4	47.6	-	-
+	5.6	78.5	21.5	-	-
+	6.26	66.7	33.3	-	-
–	0.329	62.7	19.8	8.6	8.9
–	0.626	57.2	22.4	5.8	14.7
–	1.043	60.9	18.6	5.7	14.8
–	2.083	63.4	20.9	4.5	11.2
–	3.13	71.4	24.0	2.4	2.4
–	4.2	55.4	37.5	1.8	5.4
–	4.7	61.7	15.0	5.0	18.3
–	5.2	53.6	29.3	2.1	15.0
–	5.6	50.5	23.3	5.8	20.4
–	6.26	8.0	81.6	-	10.5

* $\times 10^{-4}$ M

Legend Table 6. Percentages of cells in different stages of mitosis in roots of V. faba treated with colchicine and, or IAA. Based on data in Table 5.

Table 7

Colchicine	IAA*	Metaphases		Interphases	
		Total	% Tetraploid	Total	% Tetraploid
–	-	300	-	-	-
+	-	1000	10.6	500	15.0
+	0.329	1000	13.9	500	11.6
+	1.043	1000	7.2	-	-
+	2.083	1000	8.4	-	-
+	3.13	800	0.75	-	-
+	4.20	700	0.57	500	1.6
+	5.20	214	-	-	-
+	6.26	147	-	-	-

* $\times 10^{-4}$ M

Legend Table 7. Percentages of tetraploid cells in metaphase or in interphase in roots of V. faba, treated with colchicine and IAA. Concentrations of colchicine and IAA as in Table 2.

Table 8

control and 0.025% colchicine	$p < 0.00006$	Significant
control and the mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA	$p < 0.00006$	Significant
4.2×10^{-4} M IAA and the mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA	$p > 0.5$	Not significant
4.2×10^{-4} M IAA and 0.025% colchicine	$p < 0.00006$	Significant
0.025% colchicine and the mixture containing 0.025% colchicine and 4.2×10^{-4} M IAA	$p < 0.00006$	Significant

Legend Table 8. Probability values for significance of differences between pairs of prophase : metaphase ratios after various treatments with colchicine and, or, IAA. Probability values are based on values for F(z), which were obtained from data given in table 5.

Table 9

Colchicine	IAA x10^{-4}M	Inter-phase	Prc-phase	Meta-phase	Ana-phase	Telo-phase	MI
–	–	5627	178 (47.72)	122 (32.71)	32 (8.58)	41 (10.99)	6.22
+	–	5169	250 (30.08)	579 (69.68)	- (-)	2 (0.24)	13.85
+	0.329	5491	152 (29.86)	356 (69.94)	- (-)	1 (0.19)	8.48
+	6.26	5574	186 (43.66)	239 (56.10)	- (-)	1 (0.23)	7.10
–	0.329	5677	170 (52.63)	75 (23.22)	30 (9.29)	48 (14.86)	5.38
–	6.26	5660	206 (60.59)	82 (24.12)	12 (3.53)	40 (11.76)	5.67

Legend Table 9. Frequencies of cells in interphase and in mitosis in cells of V. faba roots treated with colchicine and, or, IAA. Percentages of cells in different stages of mitosis are given in brackets. In all treatments with colchicine the final solution contained 0.025%. The values for IAA are 0.329 and 6.26×10^{-4} M. Each determination is based on 6,000 cells; 600 cells were scored from each of 10 roots. Roots were fixed after a three hour treatment period.

Table 10

Colchicine	IAA x 10^{-4}M	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
–	–	5638	227 (62.71)	53 (14.64)	25 (6.91)	57 (15.75)	6.03
+	–	5260	265 (35.81)	475 (64.19)	- (-)	- (-)	12.33
+	0.329	5449	232 (42.11)	315 (57.17)	- (-)	(0.73)	9.18
+	6.26	5802	56 (28.28)	142 (71.71)	(- (-)	- (-)	3.30
–	0.329	5676	180 (55.56)	53 (16.36)	29 (8.95)	62 (19.14)	5.40
–	6.26	5903	52 (53.61)	19 (19.59)	6 (6.19)	20 (20.62)	1.62

Legend Table 10. Frequencies of cells in interphase and in mitosis in cells of V. faba roots treated with colchicine and, or, IAA. Percentages of cells in different stages of mitosis are given in brackets. In all treatments with colchicine the final solution contained 0.025%. The values for IAA are 0.329 and 6.26×10^{-4} M. Each determination is based on 6,000 cells; 600 cells were scored from each of 10 roots. Roots were fixed after a 24 hour period of recovery following a three hour treatment.

Table 11

Control	0.329 x 10^{-4} M IAA	6.26 x 10^{-4} M IAA	Colchicine	Colchicine and 0.329 x 10^{-4} M IAA	Colchicine and 6.26 x 10^{-4} M IAA	Value of P	
(a)							
+			+			=0.01 - 0.002	Significant
+				+		>0.50	Not significant
+					+	>0.50	Not significant
			+		+	=0.01	Significant
			+	+		=0.05 - 0.02	Not significant
+	+					=0.5 - 0.1	Not significant
+		+				>0.50	Not significant
		+	+			<0.001	Significant
	+		+			=0.002 - 0.001	Significant

Control	0.329 x 10^{-4} M IAA	6.26 x 10^{-4} M IAA	Colchicine	Colchicine and 0.329 x 10^{-4} M IAA	Colchicine and 6.26 x 10^{-4} M IAA		
(b)							
+			+			=0.02	Significant
+				+		=0.5 - 0.1	Not significant
+					+	=0.5 - 0.1	Not significant
			+		+	=0.001	Significant
			+	+		=0.5 - 0.1	Not significant
		+	+			<0.001	Significant
	+		+			=0.02 - 0.01	Significant
+		+				=0.02 - 0.01	Significant
+	+					> 0.5	Not significant

Legend Table 11. Probability values for significance of differences between pairs of mitotic indices in the controls and after various treatments with colchicine and, or IAA. The final concentrations of IAA in the solutions used are given in the table. Probability values were calculated by the $\underset{\sim}{t}$ test. The original data were given in tables 9 and 10.

(a) Roots fixed after a three hour period of treatment;

(b) Roots fixed after a three hour period of treatment, followed by a 24 hour recovery period.

Table 12

Control	0.329 x 10^{-4} M IAA	6.26 x 10^{-4} M IAA	Colchicine	Colchicine and 0.329 x 10^{-4} M IAA	Colchicine and 6.26 x 10^{-4} M IAA	value of P	
(a)							
+			+			< 0.00006	Significant
+				+		< 0.00006	Significant
+					+	< 0.00006	Significant
	+		+			= 0.14	Not significant
		+	+			= 0.91	Not significant
	+			+		< 0.00006	Significant
		+			+	< 0.00006	Significant
(b)							
+			+			< 0.00006	Significant
+				+		< 0.00006	Significant
+					+	< 0.00006	Significant
+	+					= 0.29	Not significant
+		+				= 0.14	Not significant

Legend Table 12. Probability values for significance of differences between pairs of prophase : metaphase ratios in the controls and after various treatments with colchicine and, or, IAA. Probability values are based on values for F(z), which were obtained from data given in tables 9 and 10. The final concentration of colchicine in the solutions used was 0.025%. The final concentrations of IAA in the solutions used are given in the table. Treatment was for three hours.

(a) Roots fixed after a three hour period of treatment.

(b) Roots fixed after a three hour period of treatment followed by a 24 hour period of recovery.

Table 13a. (Control)

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
0	5702	157 (52.7)	71 (23.8)	18 (60.0)	52 ((4.97)	4.97
27	5671	158 (48.0)	77 (23.4)	30 (9.1)	64 (19.50)	5.48
54	5683	221 (69.7)	45 (14.2)	12 (3.8)	39 (12.30)	5.28
57	5616	253 (65.9)	73 (19.0)	19 (4.9)	39 (10.20)	6.40
81	5586	230 (55.6)	88 (21.3)	31 (7.5)	65 (15.70)	6.90
105	5699	204 (67.8)	53 (17.6)	12 (3.9)	32 (10.60)	5.00
129	5704	171 (57.8)	63 (21.3)	19 (6.4)	43 (14.50)	4.93
153	5727	142 (52.0)	74 (27.1)	11 (4.0)	46 (16.80)	4.55
177	5685	196 (62.2)	60 (19.0)	15 (4.8)	44 (13.90)	5.25
201	5731	151 (56.1)	63 (23.4)	12 (4.5)	43 (15.90)	4.48

Legend Table 13. Frequencies of cells in interphase and mitosis in cells of V. faba roots grown in the culture medium, or treated with colchicine and, or, IAA. The final concentrations in the solutions used in treatments were 0.025% colchicine and 4.7×10^{-4} M IAA. Treatment was for three hours. Each determination is based on 6,000 cells; 600 cells were scored from each of 10 roots. Percentages of cells in different stages of mitosis are given in brackets. The duration of the experiment is given in 13a; the times of treatments and fixations in all subsequent parts of this table are based on the time scale for the controls.

(a) Controls.

Table 13b

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
54	5988	6 (50.0)	5 (41.7)	- (-)	1 (8.3)	0.20
57	5866	101 (75.4)	19 (14.2)	4 (2.9)	10 (7.5)	2.23
81	5906	41 (43.6)	25 (26.6)	9 (9.6)	19 (20.2)	1.57
105	5749	155 (61.8)	44 (17.5)	19 (7.6)	33 (13.1)	4.18
129	5779	131 (59.3)	46 (20.8)	20 (9.0)	24 (10.9)	3.70
153	5716	178 (62.7)	57 (20.1)	16 (5.6)	33 (11.6)	4.73
177	5706	166 (56.5)	60 (20.4)	19 (6.5)	49 (16.7)	4.90
201	5593	251 (61.7)	92 (22.6)	21 (5.2)	43 (10.6)	6.78

Legend Table 13b. Roots treated with an IAA solution for three hours, 27 hours after the beginning of the experiment.

Table 13c

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
57	5734	177 (66.5)	51 (19.2)	12 (4.5)	26 (9.8)	4.43
81	5928	46 (63.9)	14 (19.4)	1 (1.4)	11 (15.3)	1.20
105	5774	136 (60.2)	43 (19.0)	11 (4.9)	36 (15.9)	3.80
129	5809	107 (56.0)	32 (16.8)	22 (11.5)	30 (15.7)	3.18
153	5508	322 (65.4)	92 (18.7)	26 (5.3)	52 (10.6)	8.20
177	5593	252 (61.9)	70 (17.2)	25 (6.1)	60 (14.7)	6.80
201	5677	176 (54.5)	62 (19.2)	28 (8.7)	57 (17.6)	5.38

Legend Table 13c. Roots treated with an IAA solution for three hours, 54 hours after the beginning of the experiment.

Table 13d

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
54	5988	6 (50.0)	5 (41.7)	- (-)	1 (8.3)	0.20
57	5969	28 (90.3)	2 (6.5)	- (-)	1 (3.2)	0.50
81	6000	- (-)	- (-)	- (-)	- (-)	0.00
105	5825	85 (48.6)	39 (22.3)	18 (10.3)	33 (18.9)	2.90
129	5889	68 (61.3)	24 (21.6)	6 (5.4)	13 (11.7)	1.85
153	5799	130 (64.7)	39 (19.4)	11 (5.5)	21 (10.4)	3.35
177	5875	72 (57.6)	31 (24.8)	2 (1.6)	20 (16.0)	2.08
201	5813q	124 (66.3)	29 (15.5)	8 (4.3)	26 (13.9)	3.10

Legend Table 13d. Roots treated with an IAA solution for three hours, 27 and 54 hours after the beginning of the experiment.

Table 13e

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
0	5702	157 (52.7)	71 (23.8)	18 (6.0)	52 (4.97)	4.97
27	5879	51 (42.1)	58 (47.9)	- (-)	12 (9.92)	2.02
54	5905	26 (28.4)	50 (52.6)	- (-)	19 (20.00)	1.58
57	5910	55 (61.1)	25 (27.8)	- (-)	10 (11.10)	1.50
81	5905	23 (24.2)	54 (56.8)	1 (1.1)	17 (17.90)	1.58
105	5931	14 (20.3)	37 (53.6)	1 (1.4)	17 (24.60)	1.15
129	5945	8 (14.5)	29 (52.7)	- (-)	18 (32.70)	0.90

Legend Table 13e. Roots treated with an IAA solution for three hours at the beginning of the experiment and 27 and 54 hours later.

Table 13f

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
57	5981	9 (47.4)	7 (36.8)	- (-)	3 (15.8)	0.30
81	5897	16 (15.5)	68 (66.0)	3 (2.9)	16 (15.5)	1.70
105	5879	46 (38.0)	48 (39.7)	2 (1.7)	25 (20.7)	2.00
129	5974	12 (46.2)	11 (42.3)	- (-)	3 (11.5)	0.43

Legend Table 13f. Roots treated with an IAA solution for three hours, at the beginning of the experiment and 27 hours later.

Table 13g

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
54	5866	42 (31.3)	56 (41.8)	1 (0.75)	35 (26.1)	2.23
57	5948	29 (55.8)	14 (26.9)	- (-)	9 (17.3)	0.87
81	5881	56 (47.1)	38 (31.9)	- (-)	25 (21.0)	1.98
105	5896	28 (26.9)	57 (54.8)	2 (1.90)	17 (16.3)	1.73
129	5948	12 (23.1)	27 (51.9)	- (-)	13 (25.0)	0.83

Legend Table 13g. Roots treated with an IAA solution for three hours, at the beginning of the experiment and 54 hours later.

Table 13h

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
57	5923	35 (45.5)	28 (36.4)	- (-)	14 (18.2)	1.28
81	5763	137 (57.8)	61 (25.7)	3 (1.3)	36 (15.2)	3.95
105	5877	41 (33.3)	50 (40.7)	1 (0.8)	31 (25.2)	2.05
129	5915	29 (34.1)	32 (37.6)	1 (1.2)	23 (27.1)	1.40

Legend Table 13h. Roots treated with an IAA solution for three hours, at the beginning of the experiment.

Table 13i

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
54	4858	163 (14.27)	979 (85.73)	- (-)	- (-)	19.00
57	5231	236 (30.70)	533 (69.30)	- (-)	- (-)	12.80
81	5759	61 (25.30)	150 (62.20)	8 (3.30)	22 (9.10)	4.00
105	5945	15 (27.30)	34 (61.80)	2 (3.60)	4 (7.30)	0.90
129	5990	6 (60.00)	3 (30.00)	- (-)	1 (10.00)	0.17
153	5985	7 (46.70)	8 (53.30)	- (-)	- (-)	0.25
177	5967	20 (60.60)	8 (24.20)	- (-)	5 (15.20)	0.55
201	5998	2 (100.00)	- (-)	- (-)	- (-)	0.03

Legend Table 13i. Roots treated with a colchicine solution for three hours, 27 hours after the beginning of the experiment.

Table 13j

Hours after the experiment began	Inter-Phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
54	5954	33 (71.7)	13 (28.3)	- (-)	- (-)	0.77
57	5972	14 (50.0)	14 (50.0)	- (-)	- (-)	0.47
81	5878	44 (36.1)	61 (50.0)	2 (1.6)	15 (12.3)	2.03
105	5990	8 (80.0)	1 (10.0)	1 (10.0)	- (-)	0.17
129	5990	5 (50.0)	4 (40.0)	- (-)	1 (10.0)	0.17
153	5997	2 (66.6)	1 (33.3)	- (-)	- (-)	0.05
177	5873	85 (66.9)	23 (18.1)	5 (3.9)	14 (11.0)	2.10
201	6000	- (-)	- (-)	- (-)	- (-)	0.00

Legend Table 13j. Roots treated with a mixture containing colchicine and IAA for three hours, 27 hours after the beginning of the experiment.

Table 13k

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
54	5954	33 (71.7)	13 (28.3)	- (-)	- (-)	0.77
57	5905	61 (64.2)	34 (35.8)	(-) (-)	(-) (-)	1.58
81	5990	8 (80.0)	2 (20.0)	(- (-)	- (-)	0.17
105	5995	3 (60.0)	1 (20.0)	- (-)	1 (20.0)	0.08
129	5953	19 (40.4)	14 (29.8)	4 (8.5)	10 (21.3)	0.80
153	5928	36 (58.1)	11 (17.7)	9 (14.5)	6 (9.7)	1.03
177	5946	28 (51.9)	12 (22.2)	2 (3.7)	12 (22.2)	0.80
201	5983	13 (76.5)	3 (17.6)	- (-)	1 (5.9)	0.28

Legend Table 13k. Roots given two treatments:

(a) 27 hours after the beginning of the treatment with a mixture containing colchicine and IAA;

(b) 54 hours after the beginning of the experiment with an IAA solution. Each treatment lasted for three hours.

Table 131

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo phase	MI
0	5702	157 (52.7)	71 (23.8)	18 (6.0)	52 (4.97)	4.97
27	5879	51 (42.1)	58 (47.9)	- (-)	12 (9.92)	2.02
54	5899	50 (49.5)	35 (34.7)	- (-)	16 (15.80)	1.68
57	5933	50 (74.6)	14 (20.9)	- (-)	3 (4.50)	1.10
81	5963	11 (29.7)	18 (48.6)	- (-)	8 (21.60)	0.60
105	5938	26 (41.9)	23 (37.1)	1 (1.6)	12 (19.40)	1.03
129	5961	8 (20.5)	20 (51.3)	- (-)	11 (28.20)	0.65

Legend Table 131. Roots given two treatments:

(a) at the beginning of the experiment with an IAA solution;

(b) 27 hours after the beginning of the experiment with a mixture containing IAA and colchicine.

Each treatment lasted for three hours.

Table 13m

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
57	5901	76 (76.8)	20 (20.2)	- (-)	3 (3.0)	1.65
81	5897	41 (39.8)	45 (43.7)	4 (3.9)	13 (12.6)	1.70
105	5889	35 (31.5)	51 (45.9)	3 (2.7)	22 (19.8)	1.85
129	5962	3 (7.9)	22 (57.9)	- (-)	13 (34.2)	0.63

Legend Table 13m. Roots given three treatments:

(a) at the beginning of the experiment with an IAA solution;

(b) 27 hours after the beginning of the experiment with a mixture containing colchicine and IAA;

(c) 54 hours after the beginning of the experiment with an IAA solution.

Each treatment lasted for three hours.

Table 13n

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
0	5702	157 (52.7)	71 (23.8)	18 (6.0)	52 (4.97)	4.97
27	5671	158 (48.0)	77 (23.4)	30 (9.1)	64 (19.50)	5.48
54	4858	163 (14.3)	979 (85.7)	- (-)	- (-)	19.00
57	5366	168 (26.5)	466 (73.5)	- (-)	- (-)	10.57
81	5957	4 (9.3)	38 (88.4)	- (-)	1 (2.30)	0.70
105	5872	(53) (41.4)	(40) (31.3)	(10) (7.8)	(25) (19.50)	1.80
129	5885	41 (35.7)	53 (46.1)	3 (2.6)	18 (15.70)	1.90
153	5873	75 (59.1)	30 (23.6)	3 (2.4)	19 (14.90)	2.10
177	5864	85 (62.5)	25 (18.4)	6 (4.4)	20 (14.70)	2.27
201	5932	44 (64.7)	9 (13.2)	5 (7.4)	10 (14.70)	1.13

Legend Table 13n. Roots given two treatments:

(a) 27 hours after the beginning of the experiment with a colchicine solution;

(b) 54 hours after the beginning of the experiment with an IAA solution.

Each treatment lasted for three hours.

Table 13o

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
0	5702	157 (52.7)	71 (23.8)	18 (6.0)	52 (4.97)	4.97
27	5879	51 (42.1)	58 (47.9)	- (-)	12 (9.92)	2.02
54	5905	22 (23.2)	48 (50.5)	- (-)	25 (26.30)	1.58
57	5896	54 (51.9)	36 (34.6)	- (-)	14 (13.50)	1.73
81	5928	21 (29.2)	39 (54.2)	3 (4.2)	9 (12.50)	1.20
105	5929	21 (29.6)	39 (54.9)	2 (2.8)	9 (12.80)	1.20
129	5762	8 (21.1)	22 (57.9)	- (-)	8 (21.10)	0.63

Legend Table 13o. Roots given three treatments:

(a) at the beginning of the experiment with an IAA solution;

(b) 27 hours after the beginning of the experiment with a colchicine solution;

(c) 54 hours after the beginning of the experiment with an IAA solution.

Each treatment lasted for three hours.

Table 13p

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
57	5876	84 (67.7)	22 (17.7)	- (-)	18 (14.5)	2.07
81	5959	18 (43.9)	18 (43.9)	1 (2.4)	4 (9.8)	0.68
105	5939	14 (22.9)	36 (5910)	1 (1.6)	10 (16.4)	1.01
129	5924	14 (18.4)	46 (60.5)	- (-)	16 (21.1)	1.27

Legend Table 13p. Roots given two treatments:

(a) at the beginning of the experiment with an IAA solution;

(b) 27 hours after the beginning of the experiment with a colchicine solution.

Each treatment lasted three hours.

Table 14

Control.	Hours after the experiment began and treatment. 54 IAA	27 IAA	27 Colchicine	27 Colchicine and IAA	27 and 54 Colchicine IAA	Value of P	
1	+	+				=0.002	Significant
2	+	+				=0.01-0.002	Significant
3	+		+			<0.001	Significant
4	+			+		=0.02-0.01	Significant
5		+		+		>0.5	Not significant
6			+	+		<0.001	Significant
7	+		+			=0.01-0.002	Significant
8			+		+	=0.05	Significant
9	+				+	=0.5-0.1	Not Significant
10	+		+			=0.5-0.1	Not Significant

Legend Table 14. Probability values for significance of difference between pairs of mitotic indices in the controls and after various treatments with colchicine and, or, IAA. Probability values were calculated by the t test. The original data were given in tables 13. The final concentration of colchicine in the solution used was 0.025%; that of IAA was 4.7×10^{-4} M. Treatment was for three hours.

Roots were fixed 54 hours after the beginning of the experiment in 1, 3, 4, 5, and 6; 81 hours after the beginning of the experiment in 2 and 10; 153 hours after the beginning of the experiment in 7 and 9; and, 177 hours after the beginning of the experiment in 8.

Table 15

Control	Colchicine	IAA	Colchicine and IAA	Value of P	
+	+			<0.00006	Significant
	+	+		$=0.0002$	Significant
	+		+	<0.00006	Significant
+			+	$=0.072$	Not Significant
		+	+	$=0.27$	Not Significant

Legend Table 15. Probability values for significance of differences between pairs of prophase : metaphase ratios in the controls and after various treatments with colchicine and, or, IAA. Probability values are based on values for F(z), which were obtained from data given in tables 13. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 4.7×10^{-4} M. Treatment was for three hours. All comparisons were made with roots fixed 27 hours after the beginning of treatment.

Table 16

Hours after the experiment began	Control	Hours after the experiment began and treatment						
		27 IAA	54 IAA	27 and 54 IAA	27 Colchicine	27 Colchicine and IAA	27 Colchicine and 54 IAA	27 Colchicine and 54 IAA
0	2.20	2.20	2.20	2.20	2.20	2.20	2.20	2.20
27	2.05	2.05	2.05	2.05	2.05	2.05	2.05	2.05
54	5.00	1.20	5.00	1.20	0.17	2.54	2.54	0.17
57	3.50	5.30	3.30	14.00	0.44	1.00	1.80	0.36
81	2.60	1.60	3.30	0.00	0.41	0.72	4.00	0.11
105	3.80	3.50	3.10	2.40	0.44	8.00	3.00	1.30
129	2.70	2.80	3.30	2.80	2.00	1.25	1.40	0.77
153	2.00	3.10	3.50	3.30	0.88	2.01	3.30	2.50
177	3.30	2.80	3.60	2.30	2.50	3.70	2.30	3.40
201	2.40	2.70	2.80	4.30	-	-	4.30	4.90

Legend Table 16. Prophase : metaphase ratios of cells of roots of V. faba grown in the culture medium, or treated with colchicine and, or, IAA. The final concentration of colchicine was 0.025% in the solutions used; that of IAA was 4.7×10^{-4} M. Treatment was for three hours. In the column headings the times given indicate when, from the beginning of the experiment, the treatment was started. Since the first treatments began 27 hours after the beginning of the experiment, values for zero and 27 hours are the same for all roots, i.e. controls and treated roots. The values in the table which are underlined are from roots with a M.I. of 0.5 or less. The results given here are from data given in tables 13.

Table 17

Hours after the experiment began	Metaphases		Total cells	Percentage polyploid metaphases
	Diploid	Tetraploid		
0	500	-	500	-
27	500	-	500	-
54	254	46	300	15.3
57	435	65	500	13.0
81	265	235	500	47.0
105	156	44	200	22.0

Legend Table 17. Percentages of tetraploid cells in metaphase in roots of V. faba, treated with a 0.025% solution of colchicine, 27 hours after the beginning of the experiment. Treatment was for three hours. These results were taken from the same roots used in table 13i.

Table 18

Hours after the experiment began	Metaphases		Total cells	Percentage polyploid metaphases
	Diploid	Tetraploid		
57	410	90	500	18.0
81	475	25	500	5.0
105	174	26	200	13.0
129	89	11	100	11.0
177	99	1	100	1.0
201	98	2	100	2.0

Legend Table 18. Percentages of tetraploid cells in metaphase in roots of V. faba. Roots were treated with -

(a) a 0.025% colchicine solution, 27 hours after the beginning of the experiment; and,

(b) a 4.7×10^{-4} M IAA solution, 54 hours after the beginning of the experiment.

Treatment with each solution was for three hours. These results were taken from the same roots used in table 13n.

Table 19

Hours after the experiment began	Metaphases		Total cells	Percentage polyploid metaphases
	Diploid	Tetraploid		
0	500	-	500	-
27	500	-	500	-
54	54	-	54	-
57	96	4	100	4.0
177	98	2	100	2.0

Legend Table 19. Percentages of tetraploid cells in metaphase in roots of V. faba. Roots were treated with -

(a) a mixture containing 0.025% colchicine and 4.7×10^{-4} M IAA, 27 hours after the beginning of the experiment; and,

(b) a 4.7×10^{-4} M IAA solution, 54 hours after the beginning of the experiment.

Treatment with each solution was for three hours. These results were taken from the same roots used in table 13k.

Table 20

Hours after the experiment began	Metaphases		Total cells	Percentage polyploid metaphases
	Diploid	Tetraploid		
57	97	3	100	3.0
81	460	40	500	8.0
177	75	25	100	25.0

Legend Table 20. Percentages of tetraploid cells in metaphase in roots of V. faba. Roots were treated with a mixture containing 0.025% colchicine and 4.7×10^{-4} M IAA, 27 hours after the beginning of the experiment. Treatment was for three hours. These results were taken from the same roots used in table 13j.

Legend Table 21. Numbers of cells in interphase and in mitosis in cells of V. faba roots. Percentages of cells in different stages of mitosis are given in brackets. Each determination is based on 20,000 cells; 1,000 cells were scored from each of 20 roots. Roots were grown as follows:

(21a) control roots grown in the culture medium;

(21b) roots treated with a 6.26×10^{-4} M solution of IAA, for three hours, at the beginning of the experiment.

Roots were fixed at various times after the end of treatment. These times of fixation are given in the tables.

Table 21a

Hours after the experiment began	Interphase	Prophase	Metaphase	Anaphase	Telophase	MI
3	18,679	827 (62.6)	226 (17.1)	103 (7.8)	165 (12.5)	6.61
8	18,617	914 (66.1)	185 (13.4)	84 (6.1)	200 (14.5)	6.90
14	19,236	502 (65.7)	106 (13.9)	24 (3.1)	132 (17.3)	3.80
26	18,995	791 (78.7)	108 (10.7)	42 (4.2)	64 (6.4)	5.00

Table 21b

Hours after the experiment began	Inter-phase	Pro-phase	Meta-phase	Ana-phase	Telo-phase	MI
3	18,926	673 (62.7)	217 (20.2)	38 (3.5)	146 (13.6)	5.40
8	19,330	371 (55.4)	189 (28.2)	11 (1.6)	99 (14.8)	3.40
14	19,910	55 (61.1)	20 (22.2)	- (-)	15 (16.7)	0.45
26	19,913	59 (67.8)	15 (17.2)	2 (2.3)	11 (12.6)	0.44

Table 22a (i)

Distance from apex, in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	at the beginning of the experiment.	-	-	-	-	-	-	27 hours after the beginning of the experiment.	-	-	-	-	-	-	54 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		18	2	-	14	1	9		37	1	-	14	2	11		8	9	1	19	-	14
- 870		18	3	-	17	-	5		51	1	-	22	-	7		25	-	1	22	2	15
-1270		15	2	-	16	-	5		34	-	1	15	-	10		34	-	-	22	2	15
-1670		9	-	1	9	-	5		28	-	-	12	-	6		17	-	2	9	1	14
-2070		7	-	-	4	-	3		23	-	-	11	-	9		11	-	1	9	1	8
-2470		4	-	-	1	-	2		9	-	-	13	-	1		5	-	-	9	-	6
-2870		3	-	-	2	-	-		8	-	-	1	-	1		3	-	-	3	-	-
-3270		2	1	-	1	-	-		3	-	-	4	1	1		2	-	-	1	-	-
-3670		1	-	-	-	-	-		6	-	-	4	-	-		-	-	-	-	-	-
-4070		-	-	-	1	-	1		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	1	-	-		1	-	-	1	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	6	-	-		-	-	-	-	-	-
-5270		-	-	-	1	-	-		1	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		-	-	-	-	-	-		-	-	-	1	-	-		-	-	-	-	-	-
Total cells		189							355							291					

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Table 22a (ii)

Distance from apex, in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	102 hours after the beginning of the experiment.	-	-	-	-	-	-	174 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		27	2	-	18	1	15		15	1	-	19	2	3
- 870		31	1	1	20	3	8		17	-	-	6	-	6
-1270		25	-	-	17	-	10		14	-	-	6	-	4
-1670		11	-	-	5	-	5		12	-	-	3	-	3
-2070		6	-	-	2	-	6		2	-	-	1	-	1
-2470		3	-	-	1	-	3		3	-	-	-	-	1
-2870		-	-	-	2	-	-		-	-	-	1	-	1
-3270		-	-	-	-	-	-		1	-	-	-	-	-
-3670		-	-	-	-	-	-		3	-	-	-	-	1
-4070		-	-	-	1	-	-		-	-	-	-	-	-
-4470		-	-	-	1	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	-	-	1
-5270		-	-	-	-	-	-		-	-	-	-	-	-
5270-10070		-	-	-	-	-	-		-	-	-	1	-	1
Total cells		225							129					

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Table 22a (iii)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	222 hours after the beginning of the experiment.	-	-	-	-	-	-	270 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		6	1	-	4	1	5		14	-	-	5	-	2
- 870		9	-	-	8	-	3		11	-	-	9	-	4
-1270		4	-	-	4	-	1		2	-	-	3	-	1
-1670		-	-	-	2	-	-		-	-	-	1	-	1
-2070		-	-	-	1	-	-		-	-	-	-	-	1
-2470		-	-	-	-	-	-		-	-	-	-	-	-
-2870		1	-	-	-	-	-		-	-	-	-	-	-
-3270		-	-	-	-	-	-		-	-	-	-	-	-
-3670		-	-	-	1	-	-		-	-	-	-	-	-
-4070		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	-	-
5270 - 10,070		-	-	-	-	-	-		-	-	-	1	-	-
Total cells					51							55		

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Legend Table 22. Orientation of metaphase, telophase and anaphase figures to the longitudinal axis of primary roots of V. faba. Mitotic figures were examined in the apical 10 mm. of the root. One, 8 μ thick, median longitudinal section was examined for each of 5 primary roots in each treatment. Roots were grown in the culture medium, or were treated with 0.025% colchicine and, or, 4.7×10^{-6} M IAA. Treatment was for three hours in each case. The apex of the root was taken as the point of the junction between the root cap and the rest of the root. Mitotic figures were scored in successive 400 μ parts of the section at distances greater than 470 μ from the apex. There were few mitotic figures in the region 5270-10,070 μ from the apex of the root. The results obtained for this region were totalled in the table. This legend applies to all the tables 22.

(a) Controls.

Table 22b (i)

Distance from apex, in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	54 hours after the beginning of the experiment.	-	-	-	-	-	-	102 hours after the beginning of the experiment.	-	-	-	-	-	-	174 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		11	-	-	15	1	13		13	5	-	18	1	7		15	-	-	11	1	8
- 870		12	-	1	27	-	11		24	-	-	24	-	6		10	2	-	6	-	3
-1270		10	-	-	13	1	1		25	1	-	15	1	6		7	-	-	9	-	2
-1670		10	-	-	6	-	1		10	-	-	17	1	7		9	-	-	2	-	1
-2070		2	-	-	1	-	1		18	-	-	2	-	7		4	-	-	2	-	2
-2470		1	-	-	1	-	1		4	-	-	1	-	2		4	-	-	1	-	1
-2870		1	-	-	-	-	-		2	-	-	-	-	1		2	-	-	-	-	-
-3270		2	-	-	-	-	-		2	-	-	-	-	2		2	-	-	2	-	-
-3670		1	-	-	-	-	-		1	-	-	3	-	1		1	-	-	-	-	-
-4070		-	-	-	-	-	-		-	-	-	-	-	-		1	-	-	-	-	-
-4470		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-5270		-	-	-	-	-	-		1	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		-	-	-	-	-	-		1	-	-	1	-	-		-	-	-	-	-	-
Total cells				144							230							108			

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Table 22b(ii)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	222 hours after the beginning of the experiment.	-	-	-	-	-	-	270 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		7	-	-	3	-	3		17	-	-	13	4	11
- 870		4	-	-	3	-	1		26	4	-	13	-	6
-1270		2	-	-	2	-	-		9	-	-	3	-	4
-1670		1	-	-	-	-	-		3	-	-	2	-	-
-2070		-	-	-	-	-	-		1	-	-	1	-	-
-2470		-	-	-	-	-	-		-	-	-	-	-	-
-2870		-	-	-	-	-	-		-	-	-	-	-	-
-3270		-	-	-	-	-	-		-	-	-	1	1	-
-3670		-	-	-	-	-	-		-	-	-	-	-	-
-4070		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	1	-	-		-	-	-	-	-	1
-4870		-	-	-	-	-	-		-	-	-	-	-	1
-5270		-	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		1	-	-	1	-	1		1	-	-	2	-	-
Total cells					30							123		

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Legend Table 22b. Roots treated with a 4.7×10^{-6} M solution of IAA, 27 hours after the beginning of the experiment.

Table 22c (i)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	27 hours after the beginning of the experiment.	-	-	-	-	-	1	54 hours after the beginning of the experiment.	-	-	-	-	-	1	102 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		-	-	-	-	-	142		4	-	-	7	-	63		5	-	-	9	2	11
- 870		-	-	-	-	-	161		2	-	-	6	-	36		4	-	-	3	-	3
-1270		-	-	-	-	-	81		3	-	1	-	-	18		3	-	-	1	-	3
-1670		-	-	-	-	-	38		-	-	-	-	-	11		1	-	-	1	-	-
-2070		-	-	-	-	-	22		-	-	-	-	-	1		-	-	-	-	-	1
-2470		-	-	-	-	-	6		-	-	-	-	-	1		-	-	-	-	-	-
-2870		-	-	-	-	-	4		-	-	-	-	-	-		-	-	-	-	-	-
-3270		-	-	-	-	-	3		-	-	-	-	-	-		-	-	-	-	-	1
-3670		-	-	-	-	-	7		-	-	-	-	-	-		-	-	-	-	-	-
-4070		-	-	-	-	-	2		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	-	-	1		-	-	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	1		-	-	-	-	-	-		-	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	-	-		-	1	-	-	-	1
5270 - 10070		-	-	-	-	-	11		-	-	-	-	-	-		1	-	-	-	-	1
Total cells		479							154							55					

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Table 22c (ii)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	4	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	174 hours after the beginning of the experiment.	-	-	-	-	-	-	222 hours after the beginning of the experiment.	-	-	-	-	-	-	270 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		-	-	-	2	2	4		-	-	-	-	-	-		16	3	-	7	3	9
- 870		1	-	-	2	-	-		-	-	-	-	-	-		3	-	-	2	-	-
-1270		-	-	-	1	-	-		-	-	-	-	-	-		-	-	-	1	-	-
-1670		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-2070		-	-	-	-	1	-		-	-	-	-	-	-		-	-	-	1	-	-
-2470		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-2870		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-3270		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-3670		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-4070		1	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-4870		2	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-5270		-	1	-	-	1	3		-	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		3	10	-	3	2	-		-	-	-	-	-	-		-	1	-	-	6	1
Total cells				39							-							53			

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Legend Table 22c. Roots treated with a 0.025% colchicine solution, at the beginning of the experiment.

Table 22d (i)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	54 hours after the beginning of the experiment.	-	-	-	-	-	-	102 hours after the beginning of the experiment.	-	-	-	-	-	-	174 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		9	-	-	4	1	18		9	1	1	3	1	14		12	2	1	7	2	9
- 870		7	-	1	1	-	13		6	-	1	1	1	3		-	1	-	2	-	-
-1270		6	-	1	2	-	7		2	-	1	1	1	-		-	-	-	-	-	-
-1670		1	-	-	-	-	1		-	-	-	-	-	-		-	-	-	-	1	1
-2070		-	-	-	-	-	-		-	-	-	-	-	2		-	-	-	-	-	-
-2470		-	-	-	-	-	-		-	-	-	-	-	2		-	-	-	-	-	-
-2870		-	-	-	-	-	-		-	-	-	1	-	2		-	-	-	-	-	-
-3270		-	-	1	-	-	-		-	-	-	-	1	-		-	-	-	-	-	-
-3670		-	-	1	-	-	-		-	1	-	-	-	-		-	-	-	-	-	-
-4070		-	-	-	-	-	-		1	-	1	-	-	2		-	-	-	-	-	1
-4470		-	-	-	-	-	-		-	1	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	-	1	-		-	-	-	-	-	1
-5270		-	-	-	-	1	-		-	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		-	-	-	-	-	8		6	2	4	1	-	15		9	2	2	6	2	3
Total cells							83							94							64

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Table 22d (ii)

Distance from apex, in μ	Time of fixation	Anaphase + Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase + Telophase 1	2	3	Metaphase 1	2	3
Root cap	222 hours after the beginning of the experiment.	-	-	-	-	-	-	270 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		6	1	1	1	-	5		-	-	-	-	-	-
- 870		5	1	-	3	-	1		-	-	-	-	-	-
-1270		5	-	-	-	-	1		-	-	-	-	-	-
-1670		1	-	-	1	-	-		-	-	-	-	-	-
-2070		1	-	-	-	-	-		-	-	-	-	-	-
-2470		-	-	-	-	-	-		-	-	-	-	-	-
-2870		-	-	-	-	-	-		-	-	-	-	-	-
-3270		-	-	-	-	-	-		-	-	-	-	-	-
-3670		-	-	-	-	-	-		-	-	-	-	-	-
-4070		-	-	-	-	-	1		-	-	-	-	-	-
-4470		-	-	-	-	-	-		-	-	-	-	-	-
-4870		1	-	-	-	-	-		-	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		-	-	-	-	1	1		-	-	-	-	-	-
Total cells						37						-		

1 - orientated so as to divide parallel to the longitudinal axis of the primary root.

2 - orientated so as to divide transversely to the longitudinal axis of the primary root.

3 - orientation could not be determined.

Legend Table 22d. Roots treated as follows:

(a) with a 0.025% colchicine solution, at the beginning of the experiment;

and, (b) with a 4.7×10^{-6} M solution of IAA, 27 hours after the beginning of the experiment.

Table 22e (i)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	27 hours after the beginning of the experiment.	-	-	-	-	-	-	54 hours after the beginning of the experiment.	-	-	-	-	-	-	102 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		11	-	1	7	-	6		12	-	1	20	1	11		10	-	-	11	1	14
- 870		11	2	1	3	-	9		15	-	-	16	1	17		19	-	-	16	-	10
-1270		5	-	2	3	-	16		12	1	-	8	1	6		20	-	-	9	-	10
-1670		5	-	-	4	-	18		17	-	-	7	1	9		17	-	-	10	-	6
-2070		4	-	1	6	-	9		14	-	-	6	-	5		7	-	-	6	-	1
-2470		7	-	-	3	-	2		4	-	-	2	-	3		9	-	-	3	-	1
-2870		7	-	-	3	-	3		-	-	-	1	-	3		3	-	-	1	-	2
-3270		3	-	-	1	-	2		3	-	-	-	-	1		5	-	-	5	-	-
-3670		1	-	-	-	-	2		-	-	-	1	-	1		3	-	-	-	-	-
-4070		-	-	-	-	-	-		1	-	-	-	-	-		2	-	-	1	-	-
-4470		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	-	-	-		2	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		4	-	-	1	-	2		-	-	-	-	-	-		-	-	-	3	-	1
Total cells					165							201							208		

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Table 22e (ii)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	174 hours after the beginning of the experiment	-	-	-	-	-	-	222 hours after the beginning of the experiment	-	-	-	-	-	-	270 hours after the beginning of the experiment	-	-	-	-	-	-
0- 470		11	1	-	6	-	4		20	1	1	9	-	1		8	-	-	5	1	3
- 870		18	-	-	3	-	3		6	-	-	7	-	3		2	-	-	6	-	3
-1270		9	-	-	5	-	4		8	-	-	7	-	2		1	-	-	1	-	-
-1670		3	-	-	1	-	1		6	-	-	5	-	-		-	-	-	-	-	-
-2070		3	-	-	-	-	2		-	-	-	1	-	-		-	-	-	-	-	-
-2470		-	-	-	-	-	-		-	-	-	1	-	-		-	-	-	-	-	-
-2870		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-3270		-	-	-	1	-	-		-	-	-	1	-	-		-	-	-	-	-	-
-3670		-	-	-	-	-	-		1	-	-	-	-	-		-	-	-	-	-	-
-4070		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	-	-	-		1	-	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	1		-	-	-	-	-	-		-	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		-	-	-	-	-	-		-	-	-	1	-	-		-	-	-	-	-	-
Total cells		76							84							30					

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Legend Table 22e. Roots treated with a 4.7×10^{-6} M solution of IAA, at the beginning of the experiment.

Table 22f (i)

Distance from apex, in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	54 hours after the beginning of the experiment.	-	-	-	-	-	-	102 hours after the beginning of the experiment.	-	-	-	-	-	-	174 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		13	2	-	14	1	11		10	1	-	9	-	2		14	2	-	5	2	5
- 870		15	-	-	18	-	15		14	-	-	7	1	5		15	-	-	13	-	4
-1270		13	-	-	23	1	18		19	-	-	7	-	8		15	-	-	6	-	2
-1670		12	-	-	15	1	12		10	-	-	3	-	7		4	-	-	3	-	2
-2070		14	1	-	8	-	8		4	-	-	-	-	3		5	-	-	2	-	-
-2470		8	-	-	5	-	6		4	-	-	1	-	2		1	-	-	1	-	-
-2870		2	-	-	1	-	-		-	-	-	2	-	1		1	-	-	-	-	-
-3270		2	-	-	2	-	1		2	-	-	2	-	-		-	-	-	-	-	-
-3670		-	-	-	1	-	-		4	-	-	1	-	-		-	-	-	1	-	-
-4070		-	-	-	-	-	1		1	-	-	1	-	-		-	-	-	-	-	-
-4470		2	-	-	1	-	3		-	-	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		-	-	-	-	-	-		1	-	-	-	-	1		-	-	-	-	-	-
Total cells		250							133							103					

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Table 22f (ii)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	222 hours after the beginning of the experiment.	-	-	-	-	-	-	270 hours after the beginning of the experiment.	-	-	-	-	-	-
0-470		4	1	-	2	2	5		3	-	-	4	-	1
- 870		2	-	-	4	2	1		4	-	-	2	-	1
-1270		1	-	-	2	-	1		-	-	-	-	-	-
-1670		-	-	-	-	-	-		-	-	-	-	-	-
-2070		-	-	-	-	-	-		-	-	-	-	-	-
-2470		-	-	-	-	-	-		-	-	-	-	-	-
-2870		-	-	-	-	-	-		-	-	-	-	-	-
-3270		-	-	-	-	-	-		-	-	-	-	-	-
-3670		-	-	-	-	-	-		-	-	-	-	-	-
-4070		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	-	-
5270-10070		-	-	-	-	-	-		1	-	-	1	1	1
Total cells		27							19					

1 = orientated so as to divide parallel to the longitudinal axis of the primary root

2 = orientated so as to divide transversely to the longitudinal axis of the primary root

3 = orientation could not be determined

Legend Table 22f. Roots treated as follows:

(a) with a 4.7×10^{-6} M solution of IAA, at the beginning of the experiment;

and, (b) with a 4.7×10^{-6} M solution of IAA, 27 hours after the beginning of the experiment.

Table 22g (i)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	27 hours after the beginning of the experiment.	-	-	-	-	-	-	54 hours after the beginning of the experiment.	-	-	-	-	-	1	102 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		1	-	-	-	-	360		1	-	-	3	-	35		-	-	-	3	2	2
- 870		2	-	-	-	-	389		4	-	3	3	1	51		2	1	-	-	-	1
-1270		1	-	1	-	-	304		9	-	1	8	-	23		-	-	-	1	-	1
-1670		-	-	-	-	-	228		1	-	-	-	-	21		-	-	-	1	-	-
-2070		2	-	-	-	-	158		-	-	-	1	-	8		-	-	-	-	-	-
-2470		-	-	-	-	-	89		1	-	1	-	1	4		-	-	-	-	1	1
-2870		-	-	-	-	-	27		-	-	-	-	-	-		1	1	-	1	-	3
-3270		-	-	-	-	-	23		-	-	-	-	-	-		-	-	-	-	-	-
-3670		-	-	-	-	-	8		-	-	-	-	-	-		-	1	-	-	-	1
-4070		-	-	-	-	-	1		-	-	-	-	-	-		-	-	1	1	-	-
-4470		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	-	-	-		1	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
5270 - 10070		-	-	-	-	-	16		1	-	2	1	-	11		2	-	-	1	1	1
Total cells		1,610							196							32					

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Table 22g (ii)

Distance from apex, in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	174 hours after the beginning of the experiment.	-	-	-	-	-	-	222 hours after the beginning of the experiment.	-	-	-	-	-	-	270 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470μ		12	3	-	4	-	1		3	-	-	4	1	3		7	1	-	5	-	2
- 870		5	-	-	2	-	1		-	-	-	-	-	-		7	-	-	5	-	2
-1270		-	-	-	2	1	-		-	-	-	-	-	-		1	-	-	-	-	-
-1670		-	1	-	-	-	-		-	-	-	-	-	-		-	-	-	-	1	-
-2070		-	-	-	1	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-2470		-	-	-	-	-	1		-	-	-	-	-	-		-	-	-	-	-	-
-2870		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-3270		-	1	-	-	-	-		-	-	-	-	-	1		-	-	-	1	-	-
-3670		-	-	-	-	-	-		1	-	-	-	-	-		-	-	-	-	1	-
-4070		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	-	-	-		2	2	-	-	-	4		-	-	-	-	-	-
-4870		-	-	-	-	-	-		3	1	1	1	1	-		-	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	1	-		-	-	-	-	-	2
5270 - 10070		1	-	-	-	-	-		1	5	-	-	2	-		-	-	-	-	-	-
Total cells					36							37							35		

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Legend Table 22g. Roots treated with a solution containing 0.025% colchicine and 4.7×10^{-6} M IAA, at the start of the experiment.

Table 22h (i)

Distance from apex, in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	54 hours after the beginning of the experiment.	-	-	-	-	-	-	102 hours after the beginning of the experiment.	-	-	-	-	-	-	174 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		2	-	-	-	1	6		5	4	-	9	3	9		1	-	-	-	2	1
- 870		2	-	-	2	-	2		-	-	-	1	-	1		1	2	-	-	-	-
-1270		1	-	-	3	-	1		-	-	-	-	1	-		-	1	-	-	-	-
-1670		-	-	-	2	-	2		-	-	-	-	-	-		-	-	-	1	-	-
-2070		-	-	-	-	-	1		-	-	-	-	-	-		-	-	-	-	-	-
-2470		-	-	-	-	-	1		-	-	-	2	-	1		-	-	-	-	-	-
-2870		-	-	-	-	-	-		1	-	-	1	-	-		-	1	-	-	-	-
-3270		-	-	-	-	-	2		-	1	-	-	1	1		-	-	-	-	-	-
-3670		-	-	-	-	-	2		-	-	-	1	-	-		-	-	-	-	-	-
-4070		-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	-	-	-		-	-	-	-	-	4		-	-	-	-	-	-
-4870		-	-	-	-	-	3		-	1	-	-	-	1		-	-	-	-	-	-
-5270		-	-	-	-	-	-		-	1	-	1	-	1		-	-	-	-	-	-
5270-10070		1	-	-	1	-	32		-	-	-	1	1	1		-	-	-	-	-	1
Total cells					67							54							11		

1 = orientated so as to divide parallel to the longitudinal axis of the primary root.

2 = orientated so as to divide transversely to the longitudinal axis of the primary root.

3 = orientation could not be determined.

Table 22h (ii)

Distance from apex in μ	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3	Time of fixation	Anaphase & Telophase 1	2	3	Metaphase 1	2	3
Root cap	222 hours after the beginning of the experiment.	-	-	-	-	-	-	270 hours after the beginning of the experiment.	-	-	-	-	-	-
0- 470		4	1	-	2	-	1		2	1	-	1	-	4
- 870		-	1	-	-	-	-		-	-	-	-	-	-
-1270		-	-	-	-	-	-		-	-	-	-	-	-
-1670		-	-	-	-	-	-		-	-	-	-	-	-
-2070		-	-	-	-	-	-		-	-	-	-	-	-
-2470		-	-	-	-	-	-		-	-	-	-	-	-
-2870		-	-	-	-	-	-		-	-	-	-	-	-
-3270		-	-	-	-	1	-		-	-	-	-	-	-
-3670		-	-	-	-	-	-		-	-	-	-	-	1
-4070		-	-	-	-	-	-		-	-	-	-	-	-
-4470		-	-	-	-	-	-		-	-	-	-	-	-
-4870		-	-	-	-	-	-		-	-	-	-	-	-
-5270		-	-	-	-	-	-		-	-	-	-	-	-
5270- 10070		-	-	-	-	-	-		-	-	-	-	-	-
Total cells		10							9					

1 = orientated so as to divide parallel to the longitudinal axis of the primary root

2 = orientated so as to divide transversely to the longitudinal axis of the primary root

3 = orientation could not be determined

Legend Table 22h. Roots treated as follows:

(a) with a solution containing 0.025% colchicine and 4.7×10^{-6} M IAA, at the beginning of the experiment;

and, (b) with a 4.7×10^{-6} M solution of IAA, 27 hours after the experiment began.

Table 23

Hours from the beginning of the experiment and treatment	μ from the apex of the root: 0	1270	2470	3670	4870	6070	7270	8470	9670	10,070	Time in hours: since last treatment	from beginning of the experiment
Control												
IAA, 0											222	222
IAA, 27											243	270
IAA, 0 and 27											195	222
											243	270
Colchicine, 0											102	102
											174	174
											222	222
											270	270
Colchicine, 0; IAA,											27	54
											147	174
											195	222
											243	270
Colchicine and IAA,											54	54
											102	102
											174	174
											222	222
Colchicine and IAA, IAA, 27											27	54
											75	102
											147	174
											195	222
											243	270

Legend Table 23. Distribution of lateral root primordia and lateral roots, and position of nearest differentiated xylem vessel elements to the root apex, in the apical 10,000 μ of primary roots of V. faba. Roots were treated with colchicine and, or, IAA. Treated and untreated roots were grown in the culture medium. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 4.7×10^{-6} M. Treatment in each case was for three hours. The times from the beginning of the experiment, when treatments began, are given in the table in hours. Each determination is based on five primary roots.

\+ Position of lateral root primordia

* Position of lateral roots

x Position of nearest differentiated xylem vessel elements to the root apex

x' Roots with disorganised apices . x^2 Roots with normal apices

APPENDIX TWO.

FIGURES.

Figure 1

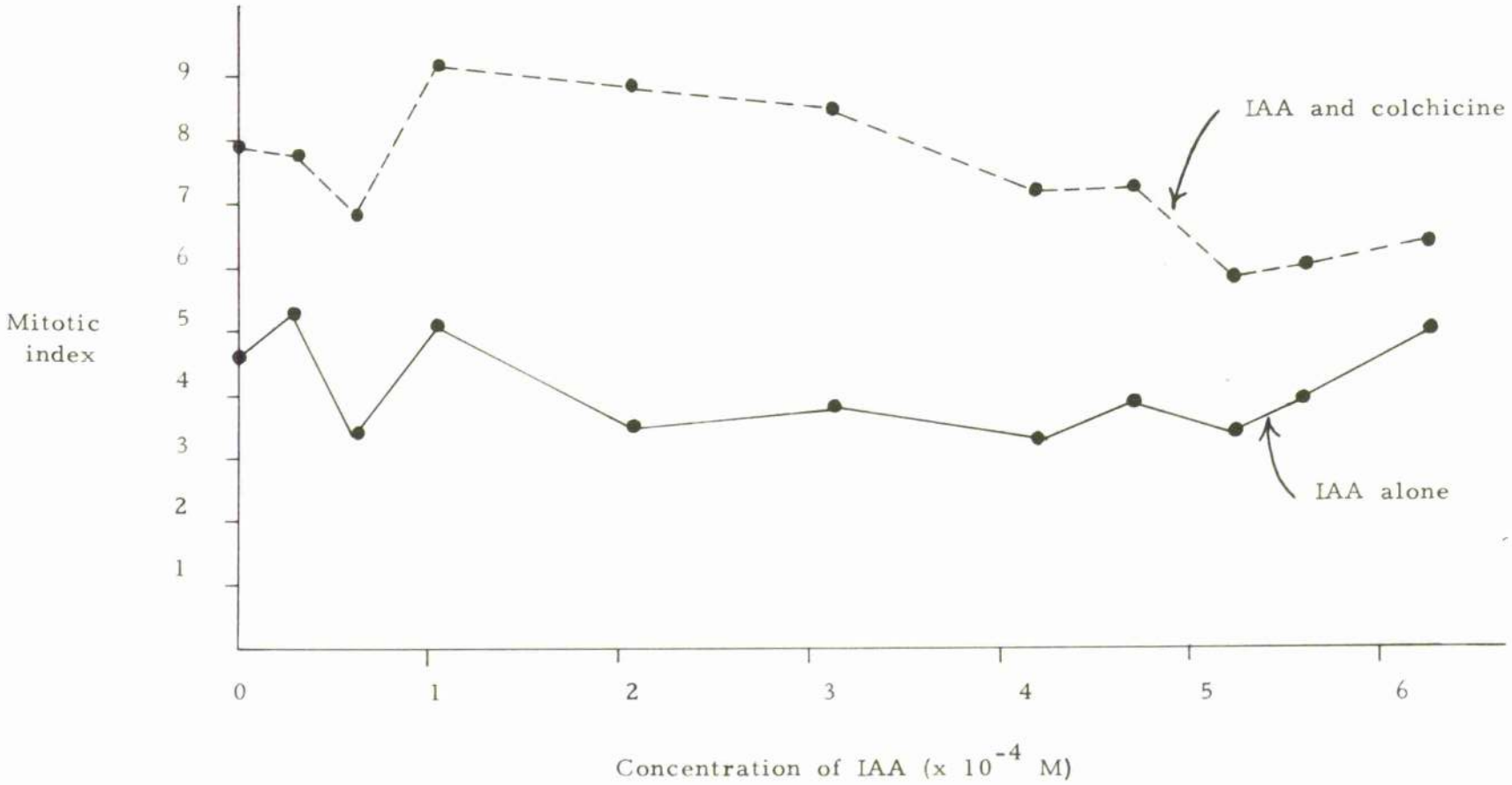

Mitotic index
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
IAA and colchicine
IAA alone
Concentration of IAA (x 10^{-4} M)

Figure 1. Mitotic indices, plotted against concentration of IAA, of roots grown in the culture medium or treated for three hours with colchicine and, or, IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 0.329 to 6.26 x 10^{-4} M. Each M.I. is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed immediately after the three hour treatment. The data on which this figure is based are from Table 2.

Figure 2

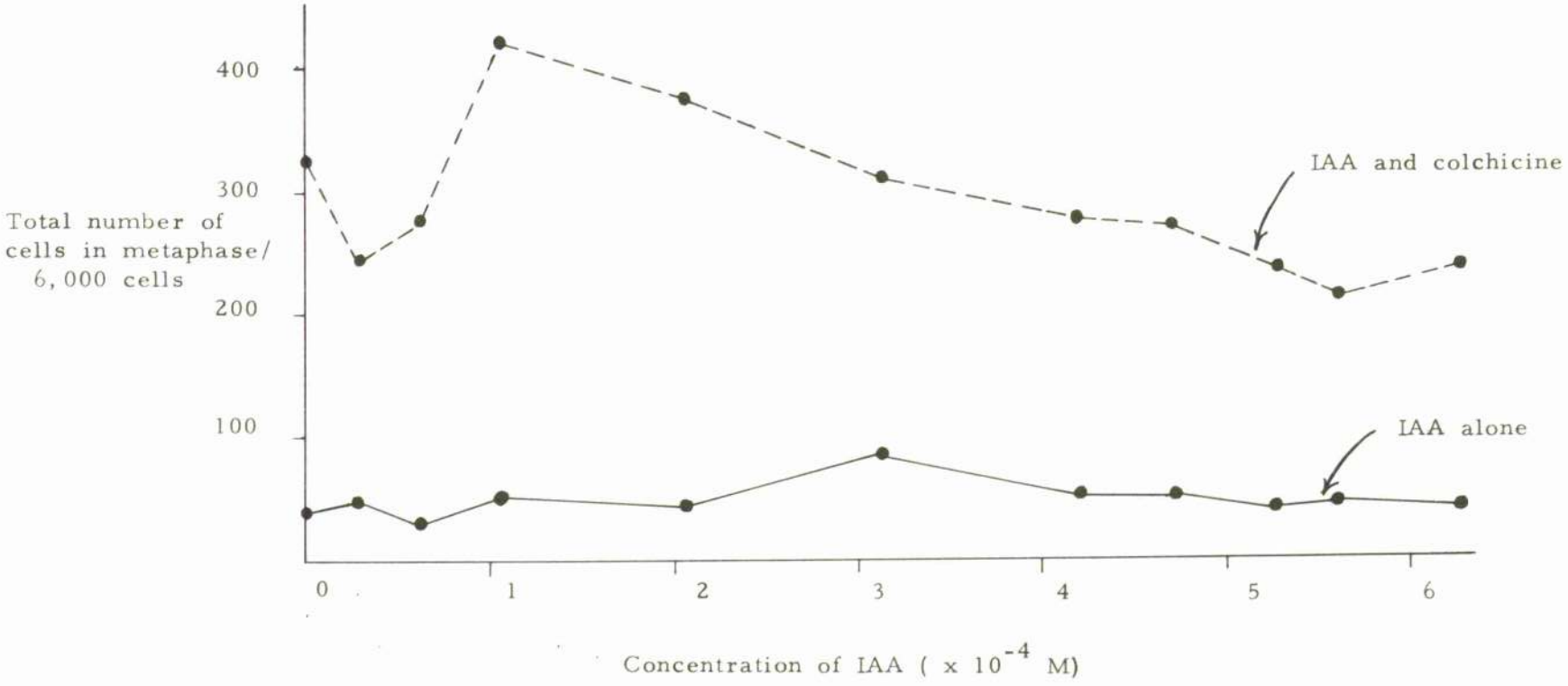

400
300
200
100
Total number of cells in metaphase/ 6,000 cells
IAA and colchicine
IAA alone
0
1
2
3
4
5
6
Concentration of IAA (x 10^-4 M)

Figure 2. Total number of metaphases/6,000 cells scored, plotted against concentration of IAA, of roots grown in the culture medium or treated for three hours with colchicine and, or, IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 0.329 to 6.26 x 10^{-4} M. Each determination is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed immediately after the three hour treatment. The data on which this figure is based are from Table 2.

Figure 3

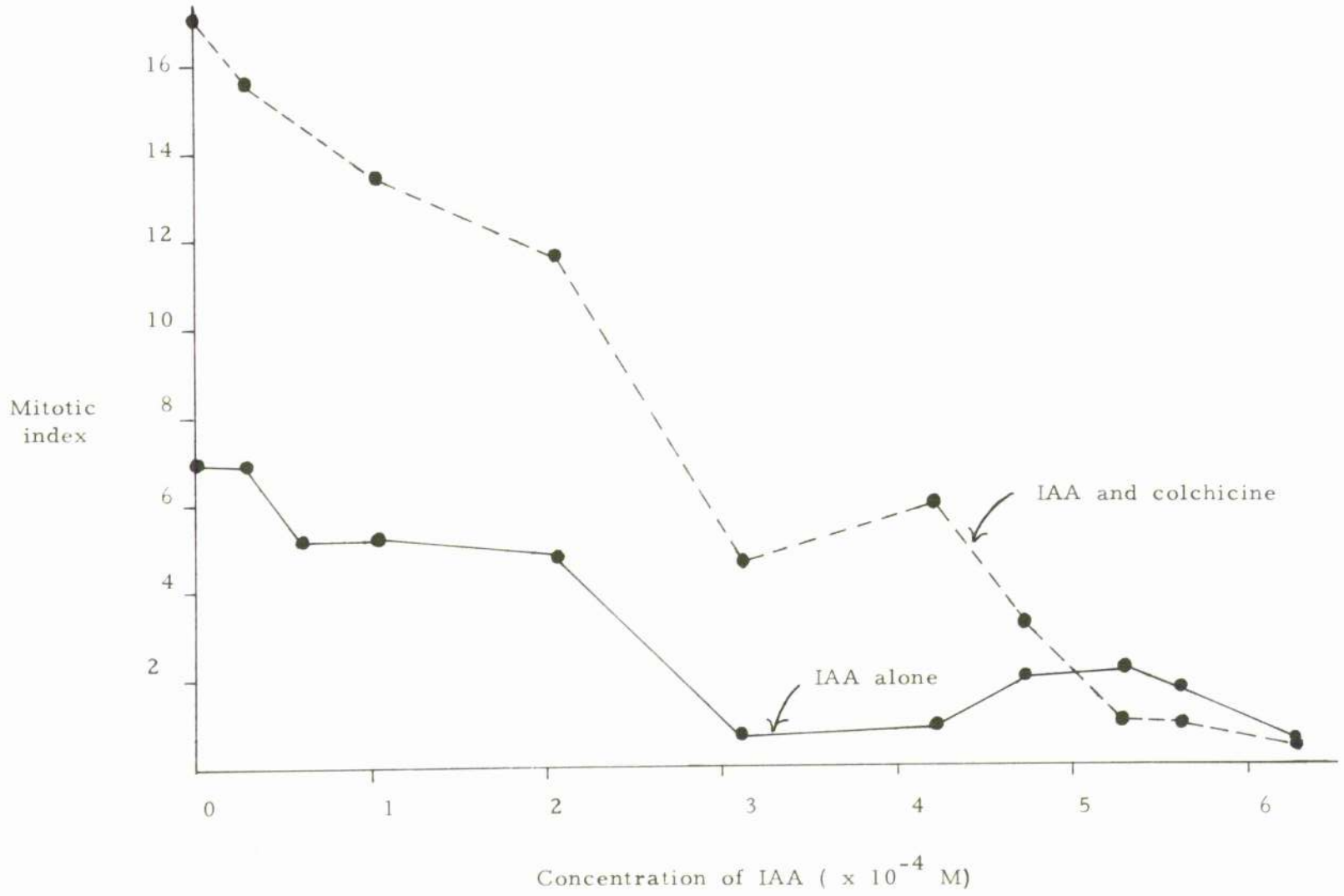

Mitotic index
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
IAA and colchicine
IAA alone
Concentration of IAA (x 10^-4 M)

Figure 3. Mitotic indices, plotted against concentration of IAA, of roots grown in the culture medium or treated for three hours with colchicine and, or, IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 0.329 to 6.26×10^{-4} M. Each M.I. is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed 24 hours after treatment ended. The data on which this figure is based are from Table 5.

Figure-4

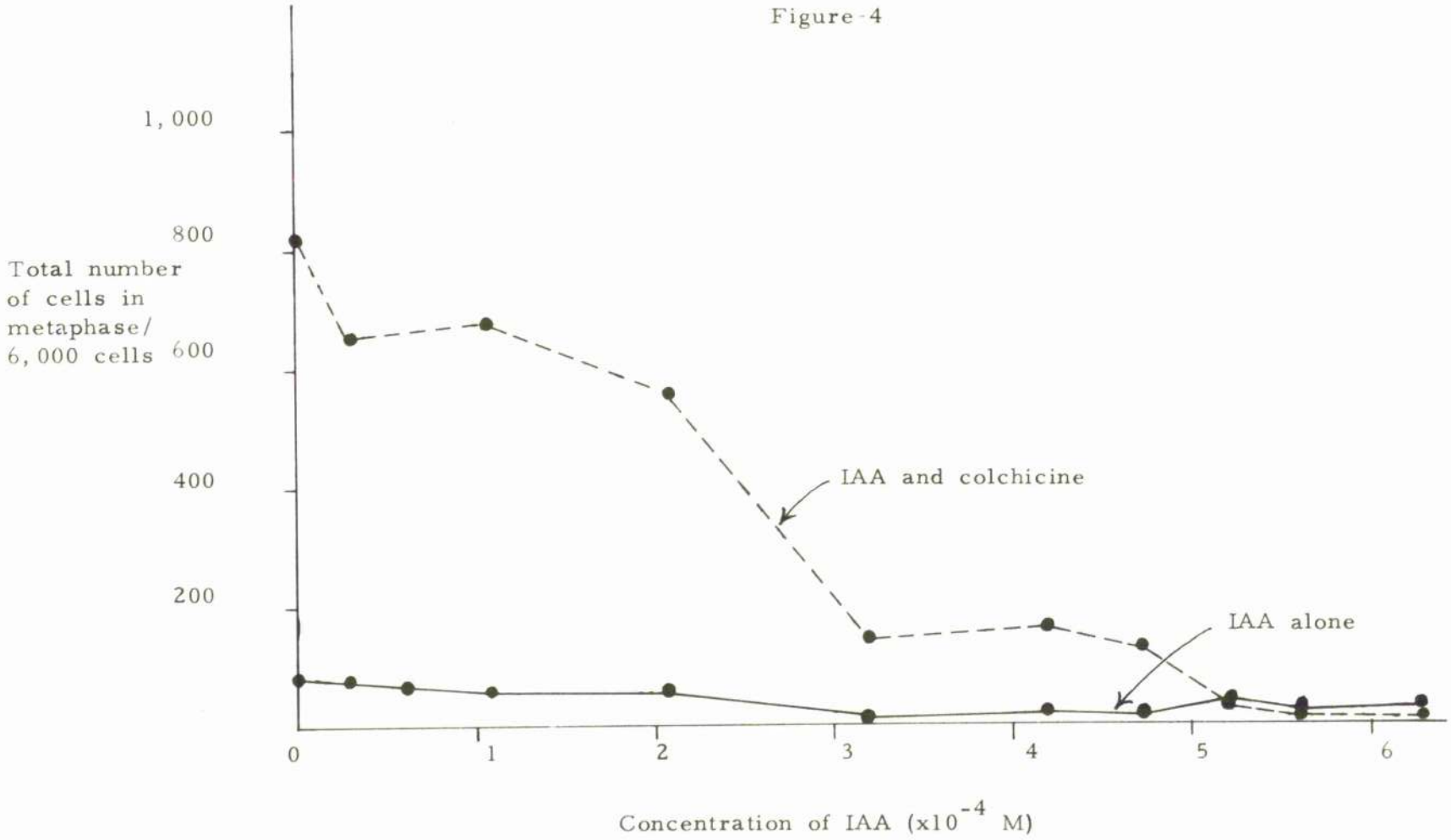

1,000
800
Total number of cells in metaphase/ 6,000 cells
600
400
200
IAA and colchicine
IAA alone
0
1
2
3
4
5
6
Concentration of IAA (x10^{-4} M)

Figure 4. Total number of metaphases/6,000 cells scored, plotted against concentration of IAA, of roots grown in the culture medium or treated for three hours with colchicine and, or, IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 0.329 to 6.26×10^{-4} M. Each determination is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed 24 hours after treatment ended. The data on which this figure is based are from Table 5.

Figure 5

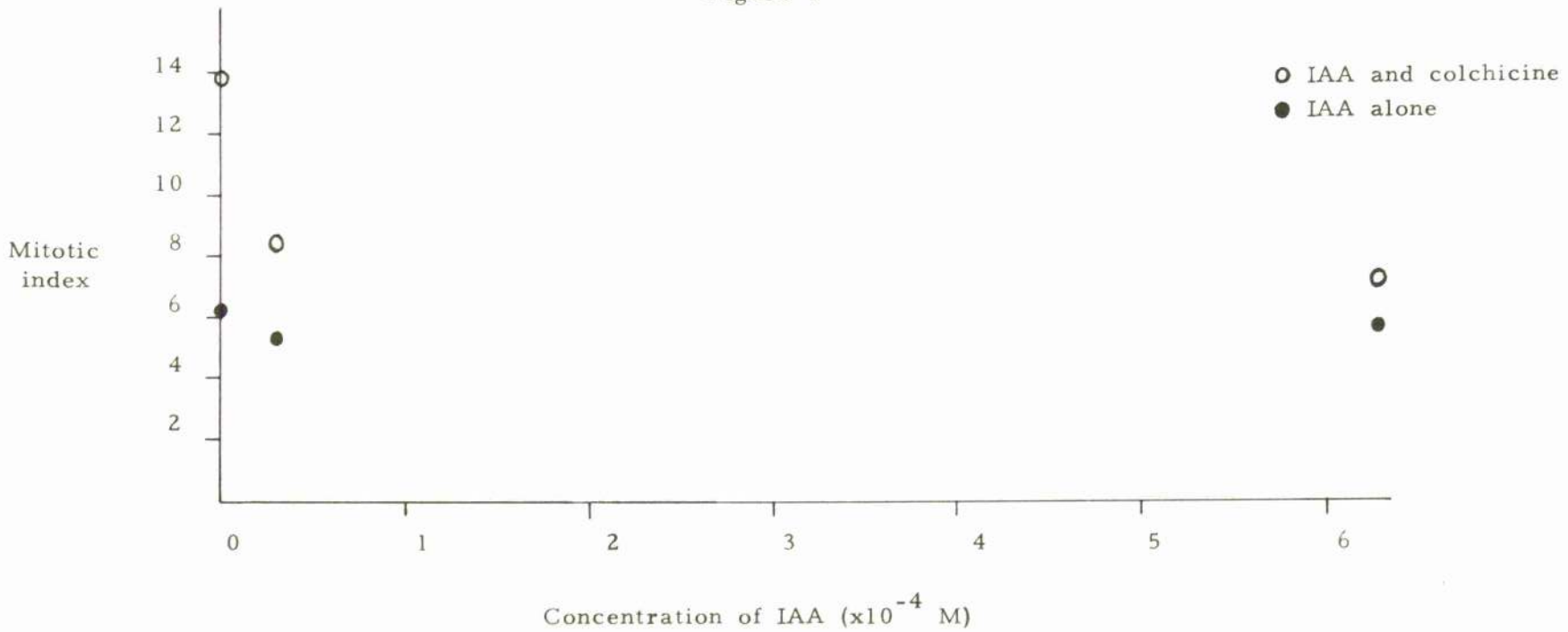

14
12
10
8
6
4
2
Mitotic index
0
1
2
3
4
5
6
IAA and colchicine
IAA alone
Concentration of IAA (x10^{-4} M)

Figure 5. Mitotic indices, plotted against concentration of IAA, of roots grown in the culture medium or treated for three hours with colchicine and, or IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 0.329 or 6.26 x 10^{-4} M. Each M.I. is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed immediately after the three hour treatment. The data on which this figure is based are from Table 9.

Figure 6

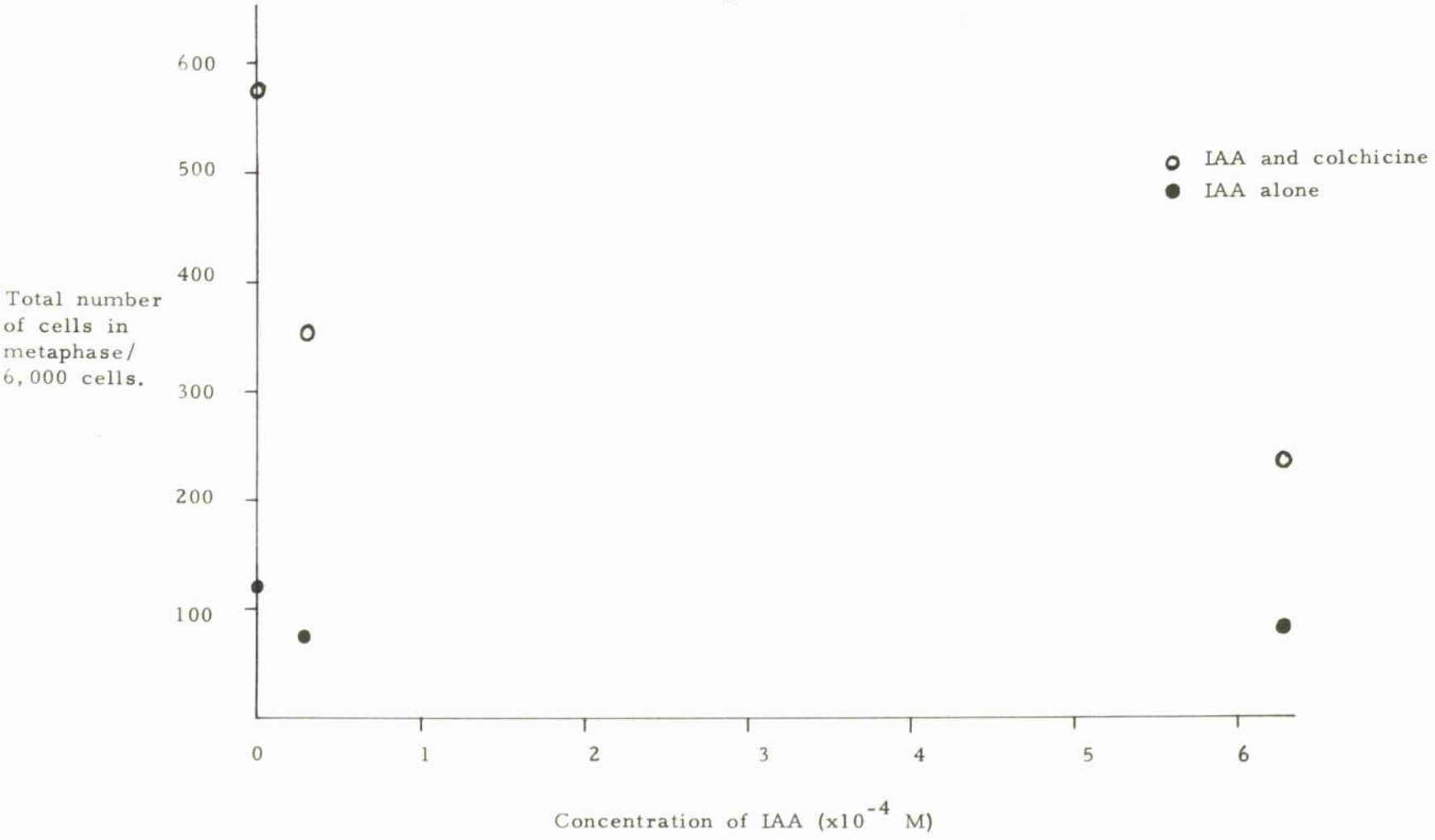

600
500
400
300
200
100
Total number of cells in metaphase/ 6,000 cells.
IAA and colchicine
IAA alone
0
1
2
3
4
5
6
Concentration of IAA (x10^{-4} M)

Figure 6. Total number of metaphases/6,000 cells scored, plotted against concentration of IAA, of roots grown in the culture medium or treated for three hours with colchicine and, or IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 0.329 or 6.26×10^{-4} M. Each determination is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed immediately after the three hour treatment. The data on which this figure is based are from Table 9.

Figure 7

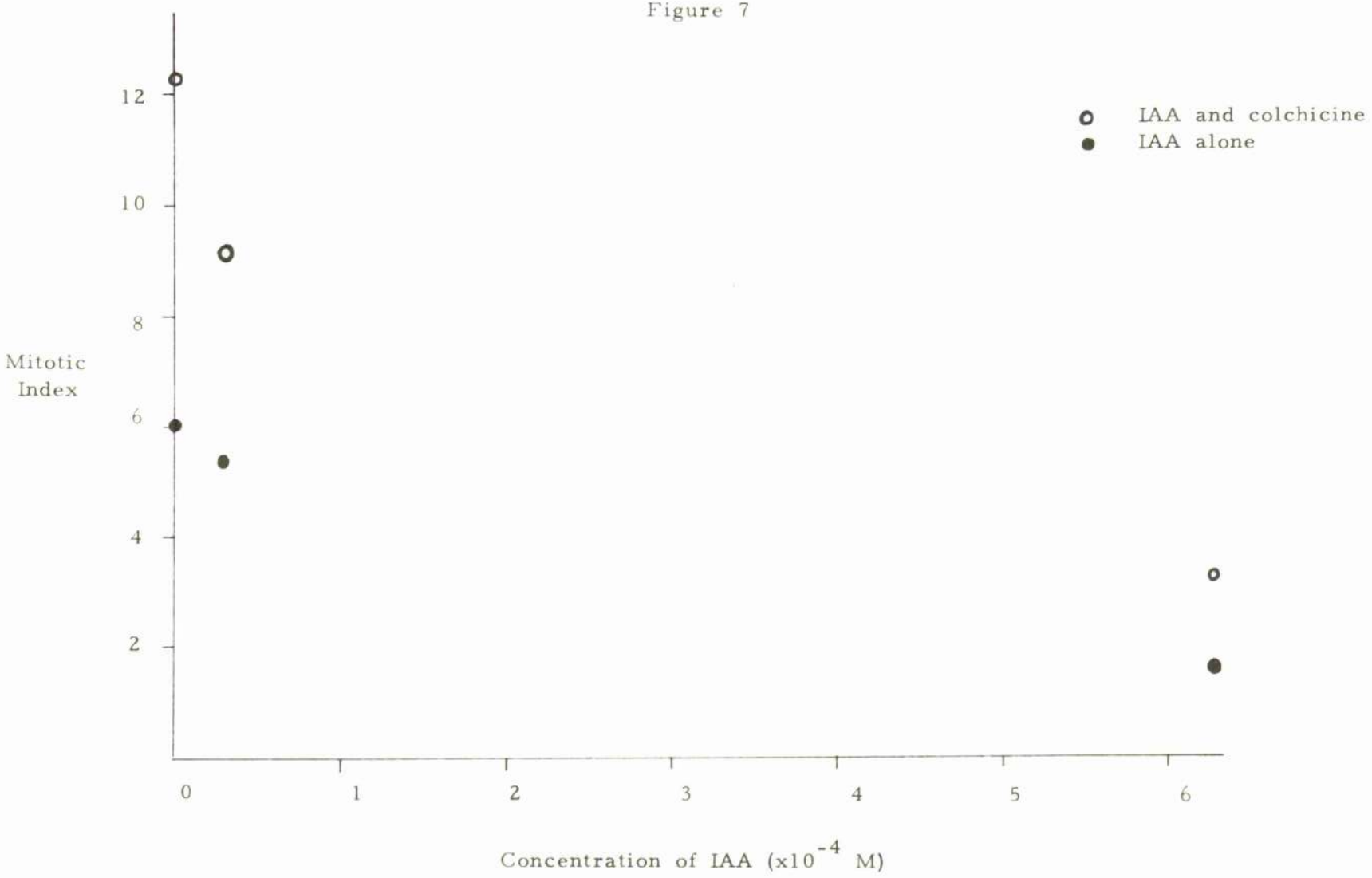

IAA and colchicine
IAA alone
Mitotic Index
12
10
8
6
4
2
0
1
2
3
4
5
6
Concentration of IAA (x10^{-4} M)

Figure 7. Mitotic indices, plotted against concentration of IAA, of roots grown in the culture medium or treated for three hours with colchicine and, or IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 0.329 or 6.26×10^{-4} M. Each MI is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed 24 hours after treatment ended. The data on which this figure is based are from Table 10.

Figure 8

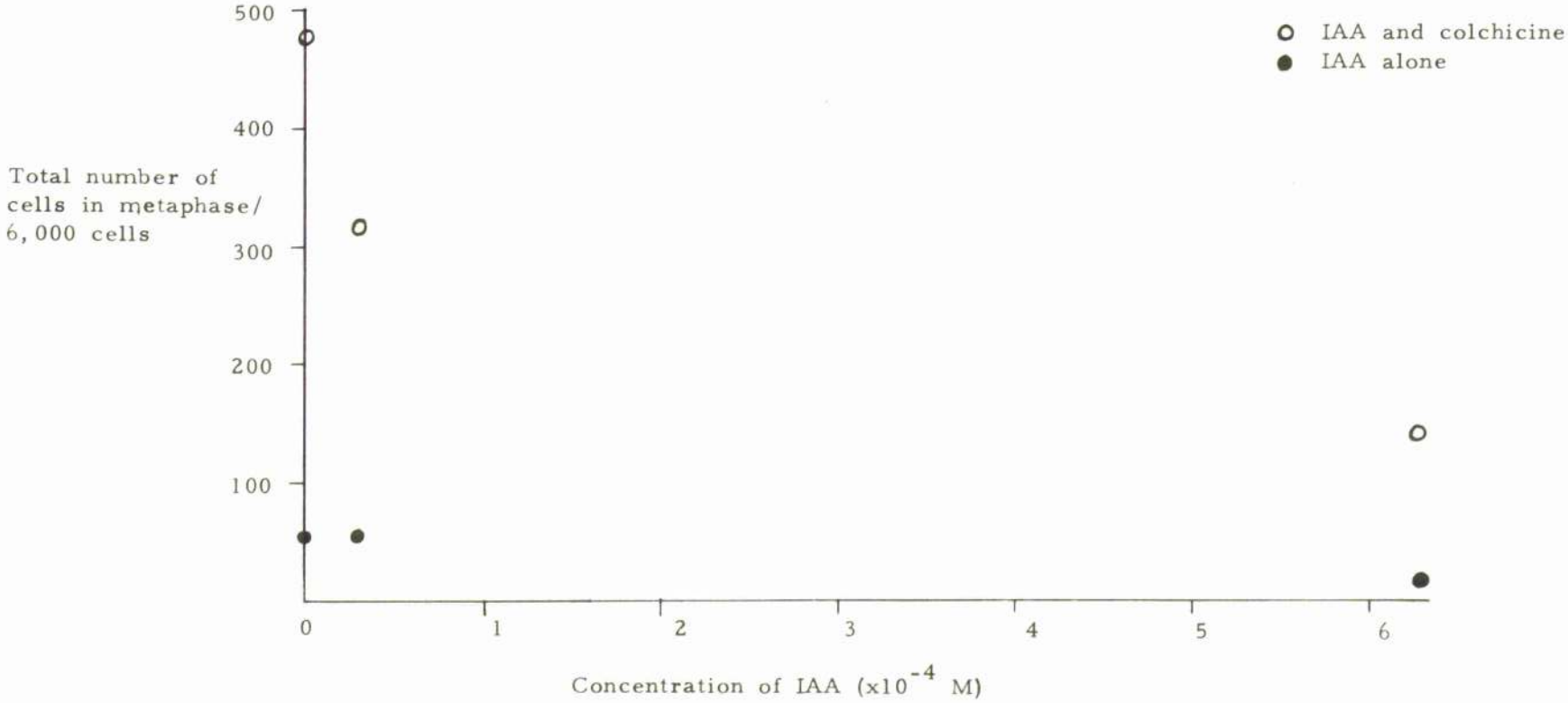

500
400
300
200
100
Total number of cells in metaphase/ 6,000 cells
IAA and colchicine
IAA alone
0
1
2
3
4
5
6
Concentration of IAA (x10^{-4} M)

Figure 8. Total number of metaphases/6,000 cells scored, plotted against concentration of IAA, of roots grown in the culture medium or treated for three hours with colchicine and, or IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 0.329 or 6.26×10^{-4} M. Each determination is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed 24 hours after treatment ended. The data on which this figure is based are from Table 10.

Figure 10

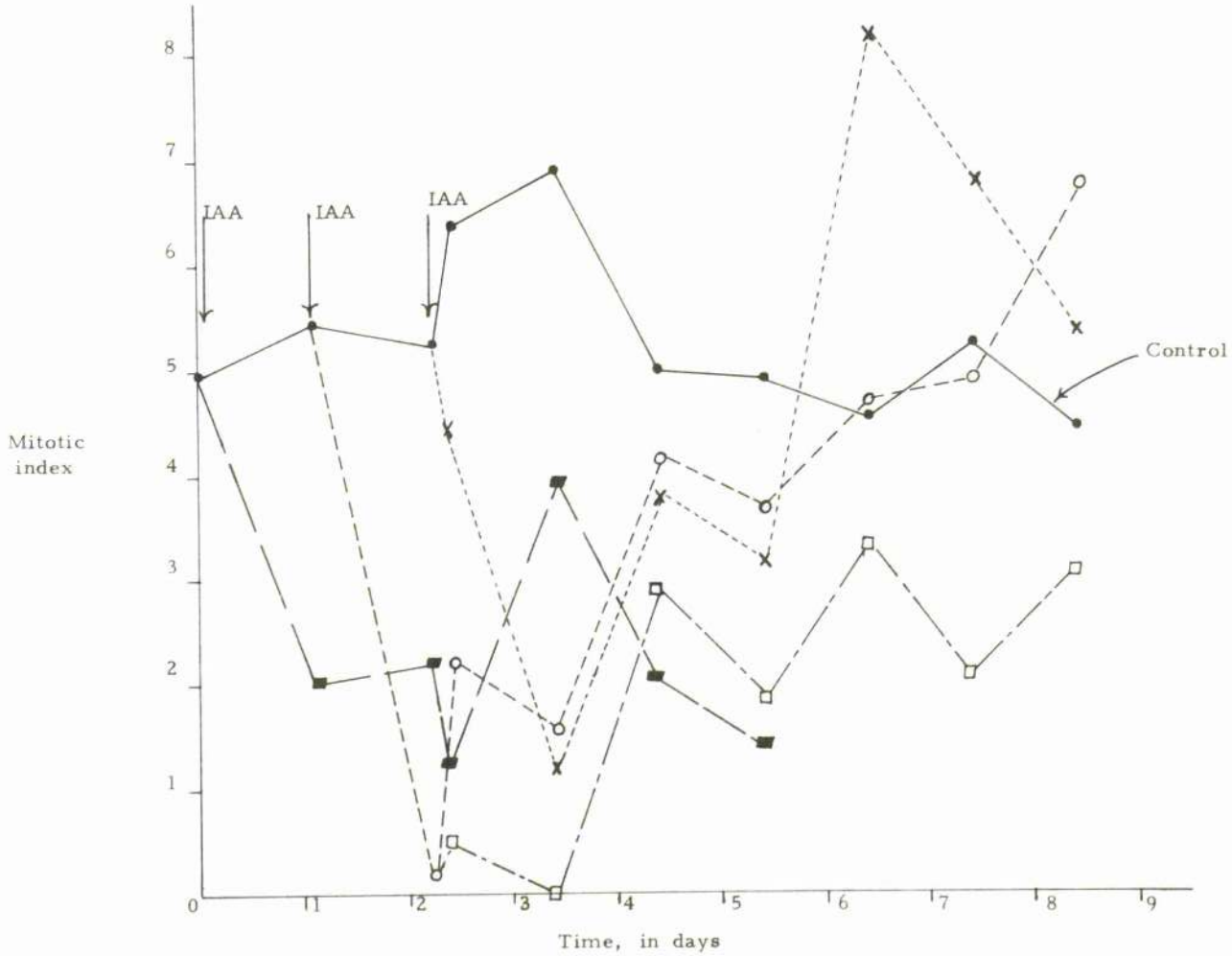


Figure 10. Mitotic indices, plotted against time, of roots grown in the culture medium or treated for three hours with a 4.7×10^{-4} M solution of IAA. Treatment was at zero, and, or, 27 and, or, 54 hours from the beginning of the experiment. Each determination is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed at the beginning of the experiment, and 27, 54, 57, 81, 105, 129, 153, 177 and 201 hours after the experiment began. The data on which this figure is based are from Tables 13(a), (b), (c), (d) and (h).

Figure 11

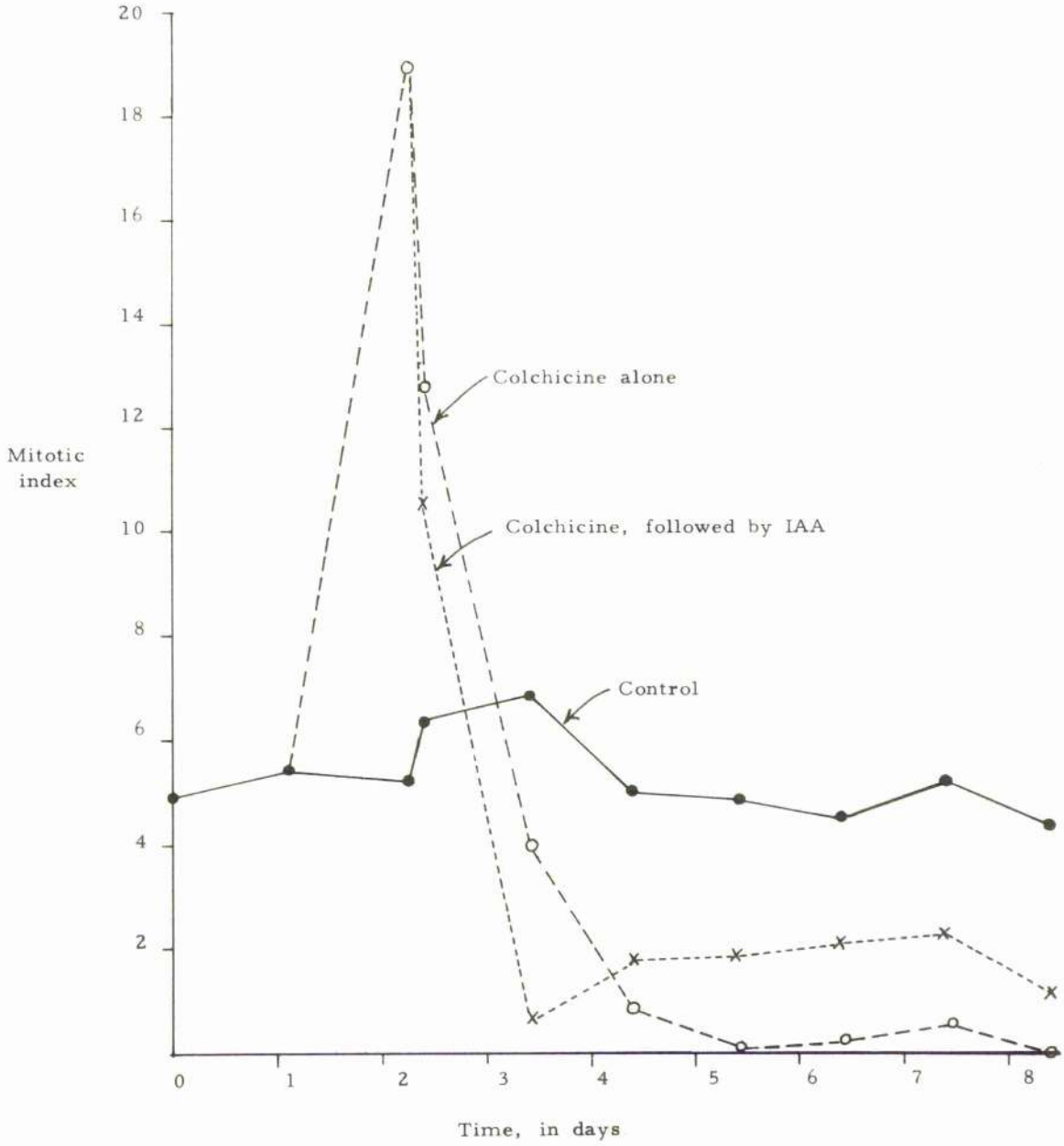

20
18
16
14
12
10
8
6
4
2
Mitotic index
Colchicine alone
Colchicine, followed by IAA
Control
0
1
2
3
4
5
6
7
8
Time, in days

Figure 11. Mitotic indices, plotted against time, of roots grown in the culture medium or treated for three hours with colchicine or colchicine followed by IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 4.7×10^{-4} M. Treatment with colchicine took place 27 hours and treatment with IAA took place 54 hours after the beginning of the experiment. Each determination is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed at the beginning of the experiment and 27, 54, 57, 81, 105, 129, 153, 177 and 201 hours after the experiment began. The data on which this figure is based are from Tables 13(a), (i) and (n).

Figure 12

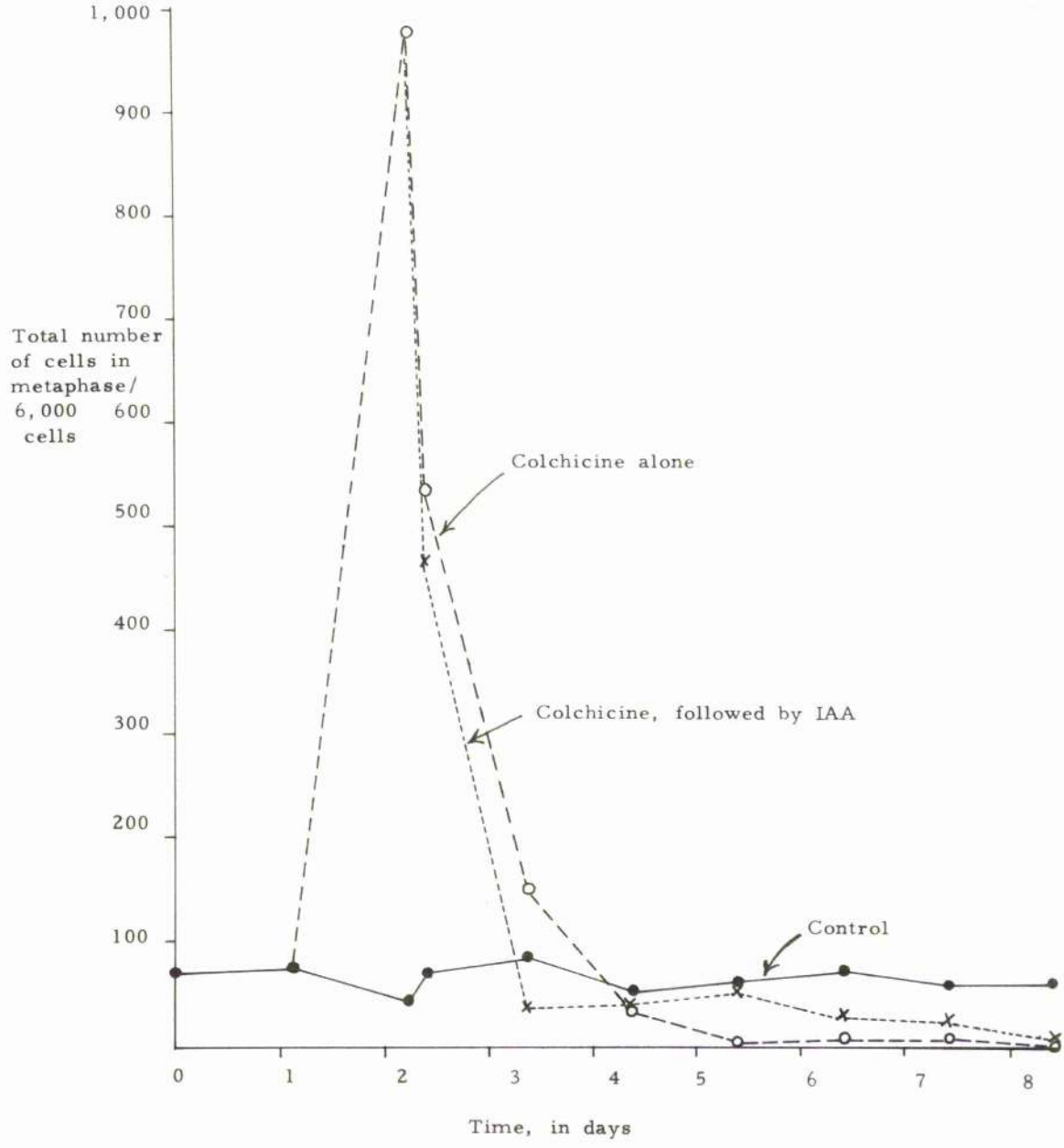
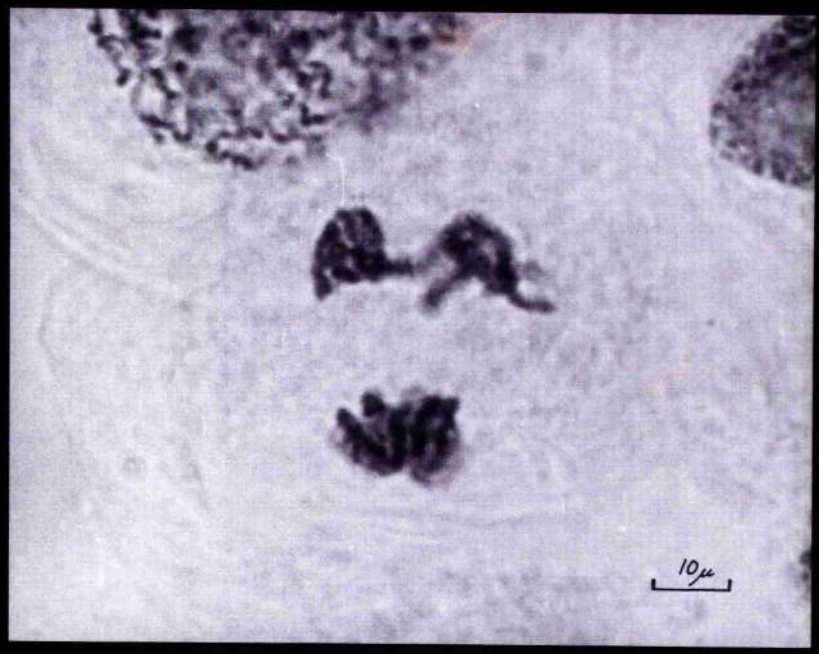

1,000
900
800
700
600
500
400
300
200
100
Total number of cells in metaphase/ 6,000 cells
Colchicine alone
Colchicine, followed by IAA
Control
0
1
2
3
4
5
6
7
8
Time, in days

Figure 12. Total number of metaphases/6,000 cells scored, plotted against time, of roots grown in the culture medium or treated for three hours with colchicine or colchicine followed by IAA. The final concentration of colchicine in the solutions used was 0.025%; that of IAA was 4.7×10^{-4} M. Treatment with colchicine took place 27 hours and treatment with IAA took place 54 hours after the beginning of the experiment. Each determination is based on 6,000 cells; 600 cells were scored from each of ten roots. Lateral roots were fixed at the beginning of the experiment and 27, 54, 57, 81, 105, 129, 153, 177 and 201 hours after the experiment began. The data on which this figure is based are from Tables 13(a), (i) and (n).

Figure 14. Three groups of chromosomes in a telophase cell in a root of V. faba. Individual chromatids cannot be made out. These roots were treated with a mixture containing 0.329×10^{-4} M IAA and 0.025% colchicine for three hours and fixed 24 hours later. The cell began to recover from the effects of colchicine treatment but a tripolar spindle was formed.

Figure 15. Five groups of chromosomes in a telophase cell in a root of V. faba. Individual chromatids cannot be made out. These roots were treated with a mixture containing 0.329×10^{-4} M IAA and 0.025% colchicine for three hours and fixed 24 hours later. The cell began to recover from the effects of colchicine treatment but a spindle with five poles was formed.


10μ

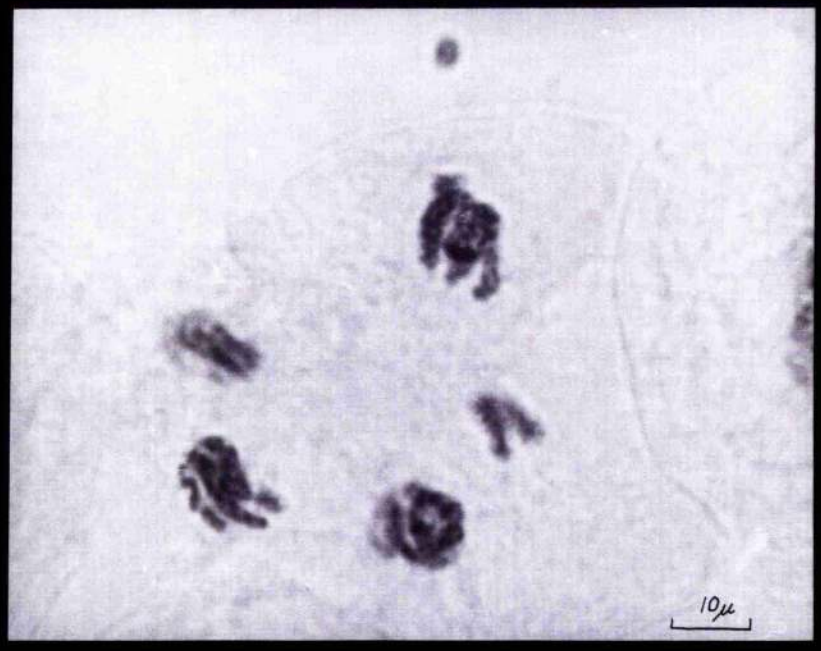
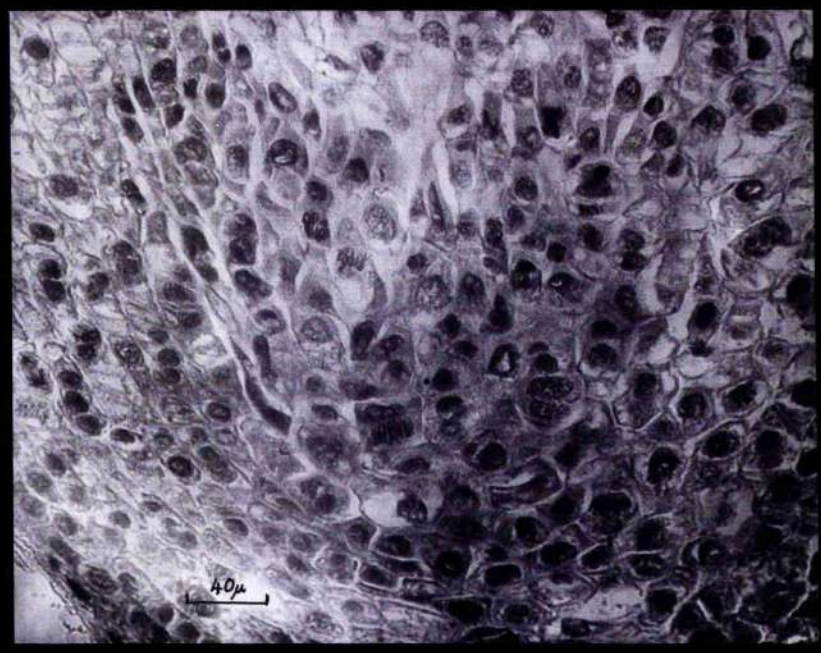

10μ

Figure 16. Cells in the root apex of V. faba 219 hours after treatment for three hours with a 0.025% colchicine solution. The cells are not arranged linearly and lateral expansion of polyploid cells has contributed to the disorganised arrangement of the cells. Sections were 8 μ thick and were stained with haemalaun.

Figure 17. Cells in the root apex of V. faba 219 hours after treatment for three hours with a 0.025% colchicine solution. Rows of polyploid and diploid cells are present in the apex. Sections were 8 μ thick and were stained with haemalaun.


40μ

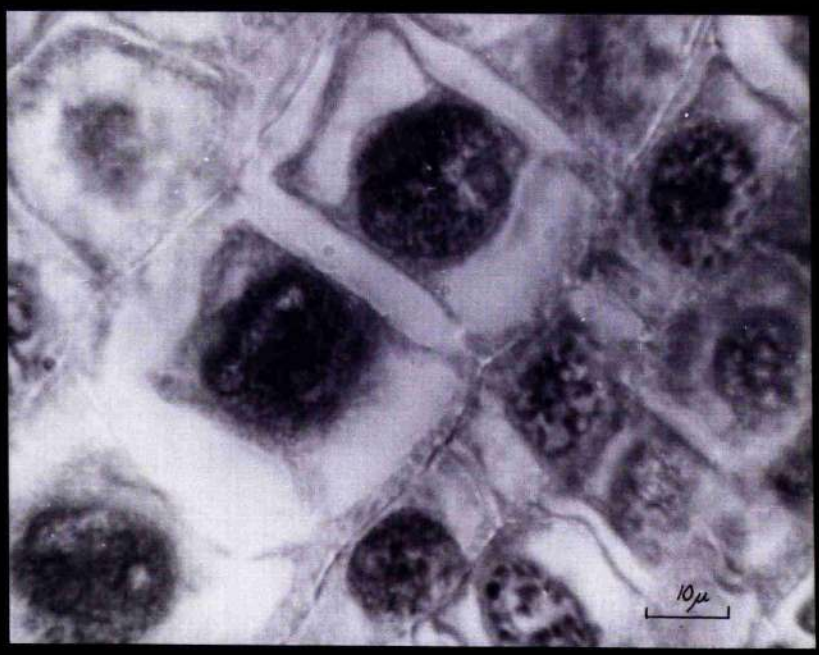
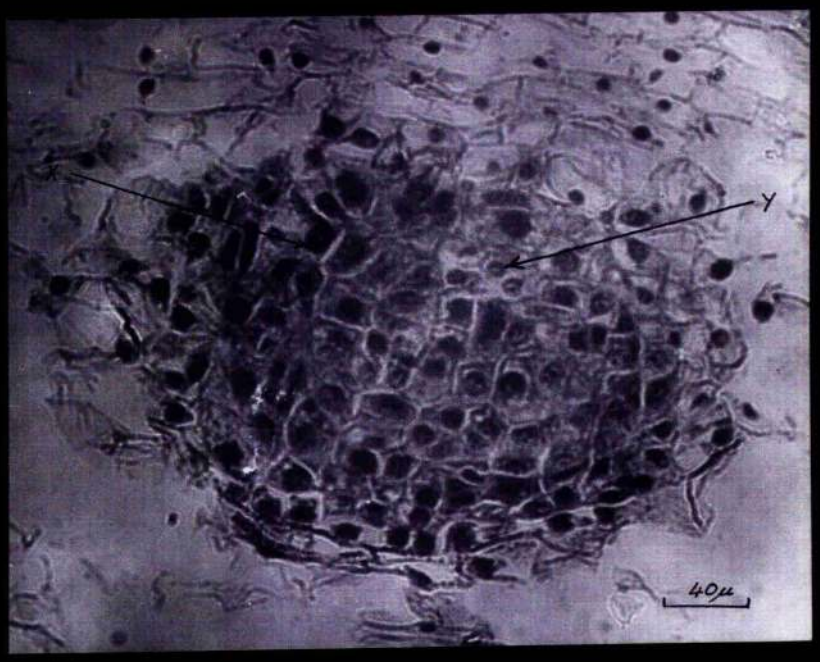

10μ

Figure 18. A lateral root primordium in a root of V. faba, 99 hours after a three hour treatment with a 0.025% colchicine solution. Polyploid cells are present in this primordium.

x = polyploid cell. y = diploid cell.

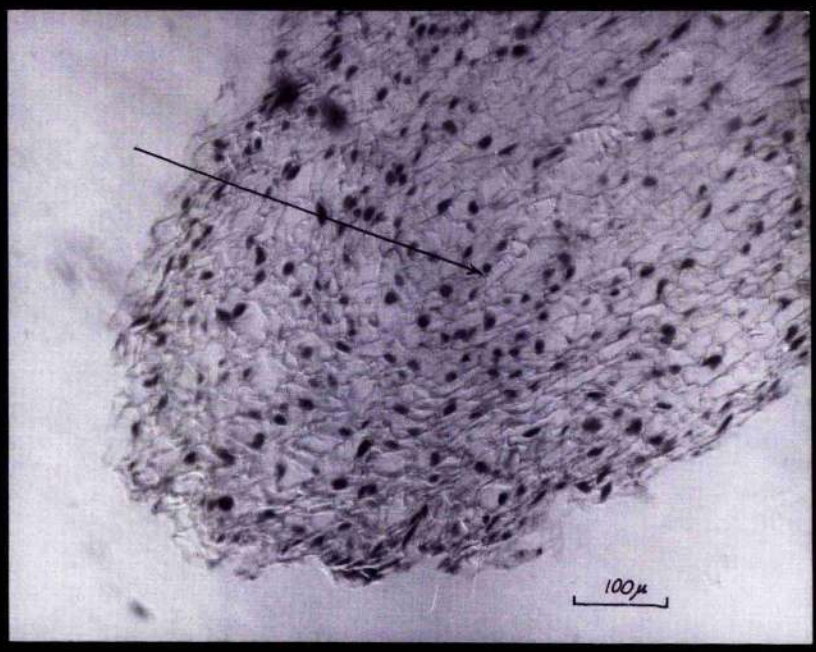

X
Y
40μ

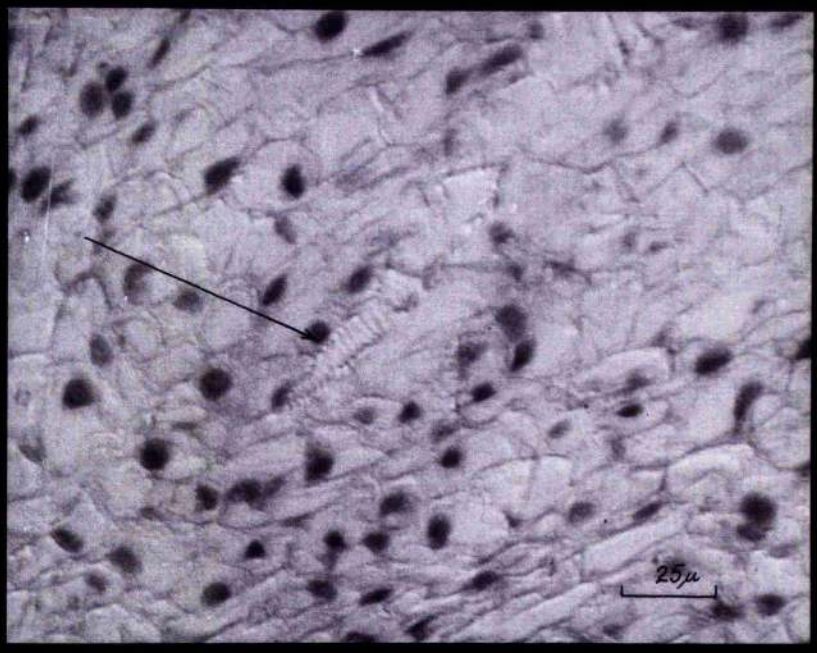
Figures 19 and 20. A xylem vessel element, indicated by an arrow, in the apical 870 μ of an apex of a root of V. faba, 8 days after treatment. Treatment was with:

i) a 0.025% colchicine solution at the beginning of the experiment and,

ii) a 4.7×10^{-6} M solution of IAA, 24 hours after colchicine treatment ended.

Treatment was for three hours in each case. Figure 20 is an enlargement of part of Figure 19, showing the xylem vessel element more clearly.


100μ


25μ